ALBURTYLAB

Leonard Wood Institute Demonstration of a Sample Preparation Method for Biological Detection Based on a Novel Membrane Fractionation Technology

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Preface

AlburtyLab, Inc. has prepared this Technical Report for the Leonard Wood Institute (LWI), under contract 16201, presenting efficiency and concentration factor results obtained for processing of DNA with a dual stage InnovaPrep© System (formally referred to as a Hydraprep). The system consisted of a cleanup InnovaPrep membrane followed by a final concentrating InnovaPrep membrane.

Dorsey Newcomb was the Program Manager for LWI. Mr. Andrew Page was the overall project leader and primary investigator for AlburtyLab, Inc. and Mrs. Pam Murowchick and Mr. Page were the authors of this report. Technical questions may be directed to Mr. Page at (816) 868-6204 or via email to apageconsult@sbcglobal.net.

APPROVED FOR ALBURTYLAB, INC.

David S. Alburty
President

December 31, 2008

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Acronyms

Bg	*Bacillus globigii* also known as *Bacillus arophaeus*
CF	Concentration factor
CFU/mL	Colony Forming Units per milliliter
Ct	Cycle threshold—defined as the number of qPCR cycles required for the fluorescent signal to cross the threshold (ie exceeds background level). Ct levels are inversely proportional to the amount of target nucleic acid in the sample (ie the lower the Ct level the greater the amount of target nucleic acid in the sample)
DNA	Deoxyribonucleic acid
kD	kiloDalton
LWI	Leonard Wood Institute
MRI	Midwest Research Institute
qPCR	Quantitative Polymerase Chain Reaction
RPM	Revolutions per minute
SD	Standard deviation
TNTC	Too numerous to count
TSA	Trypticase Soy Agar
µg/mL	Microgram per milliliter
µL	Microliter
µm	Micrometer

1. Introduction

LWI funding has been obtained to gather proof-of-concept data for passing DNA from lysed bacteria through a membrane and for concentrating DNA in the concentrator cell. AlburtyLab has data showing high efficiency (~99%) for passing Bovine serum albumin through an appropriate membrane and data showing high efficiency (~60%) for concentrating 0.03 μm microspheres with the system. Data showing similar efficiencies for DNA would significantly improve the quality of future SBIR submissions.

AlburtyLab specifically requested this funding for the purpose of performing quantitative testing to determine the efficiency data for (1) passage of DNA through a cleanup InnovaPrep© membrane and (2) concentration of DNA in a final concentrating InnovaPrep membrane.

AlburtyLab contracted with Midwest Research Institute (MRI), of Kansas City, Missouri, to prepare a stock of *Bacillus globigii* (Bg), American Type Culture Collection (ATCC) 9733, available from the Critical Reagents Program as ANG-BACI051, also known as *Bacillus atrophaeus*. MRI lysed the stock with a standard, commercially available bead beater (BioSpec Mini). The lysed stock was delivered to AlburtyLab for processing with the dual stage InnovaPrep system. All testing was performed by AlburtyLab personnel at the downtown engineering laboratory located in Drexel, Missouri. All resulting samples obtained after feed processing through the dual-stage InnovaPrep system were returned to MRI for analysis by quantitative PCR (qPCR). Detailed testing procedures are given in Section 2. Testing results are presented in Section 3. Section 4 contains discussion and conclusions.

A total of 15 runs were conducted at two feed titer test levels. In the text, the individual concentrating cycles performed by the dual-stage InnovaPrep system are referred to as runs and the series of runs performed during a single feed titer level is referred to as a test.

2. Testing Procedures

During August of 2008 two InnovaPrep units were set up and preliminary polystyrene microsphere test runs were performed to verify that the systems were operating correctly. Figure 2-1 contains a photo of the units.

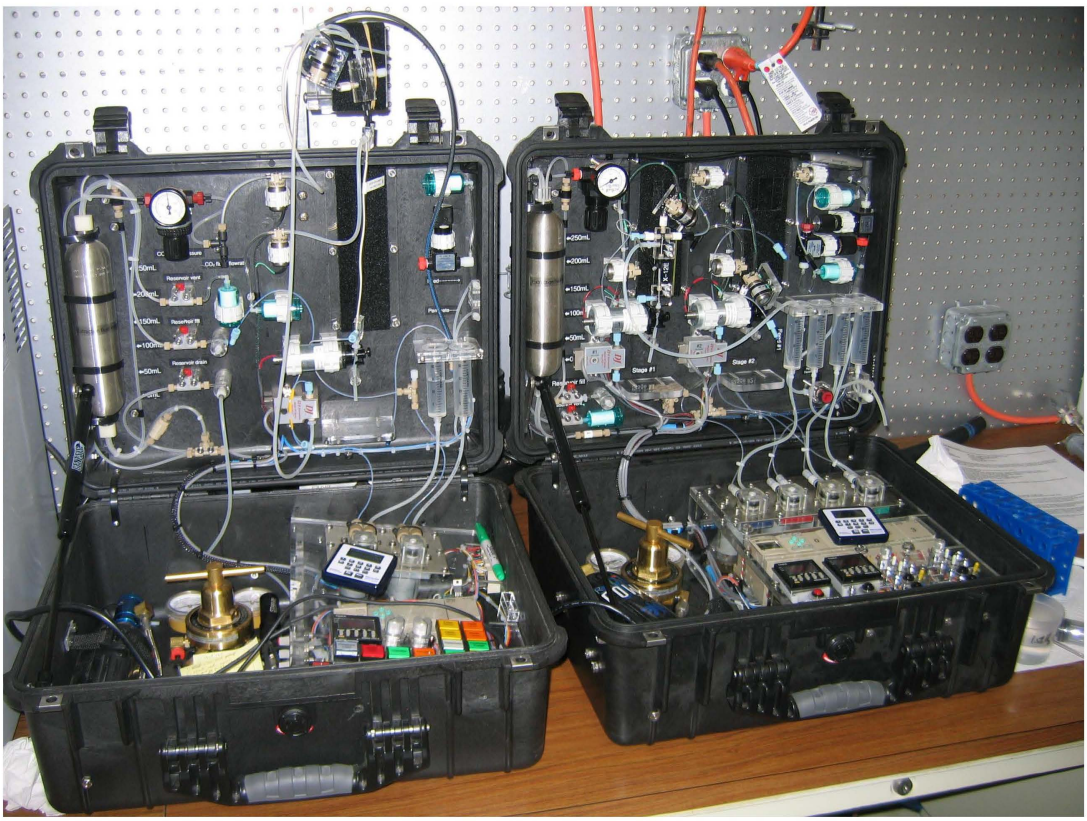

Figure 2-1. Photograph of Test Setup

2.1 System Cleaning and Passivation

Following the polystyrene microsphere test runs all fluidics paths in the InnovaPrep systems were cleaned with 1.5% hydrogen peroxide. The hydrogen peroxide was pumped into the system and allowed to stand for a minimum of 30 minutes. The hydrogen peroxide was then pumped out and copious amounts of DNA grade water were pumped through the systems to flush any remaining hydrogen peroxide from the system. The extraction fluid reservoirs were also cleaned with hydrogen peroxide and rinsed with DNA grade water prior to filling.

For very dilute protein solutions (< 10 µg/mL), concentrate recovery in ultrafiltration devices has been found to not be quantitative. Strategies that reduce adsorptive loss of proteins on surfaces

are either based on pretreatment of the surface to fill the exposed binding sites or by changes in the composition of the solution, usually by addition of protein (often albumin), detergents, or salts. Pall Corporation reports that the recovery of proteins from dilute solutions can be improved by passivation. Studies performed by Pall have shown that, in most cases, pretreating (passivating) the device before concentration of dilute protein solutions can improve recovery.[1]

The following procedure was used to passivate the internal surfaces of the InnovaPrep systems prior to processing the lysed bacteria.

1. Added approximately 5 mL of the sterile passivation solution, 5% Triton-X in distilled water, to each of the feed reservoirs of the InnovaPrep systems.
2. Pumped the passivation solution through the large pore membrane to the permeate side and through the small pore membrane to the sample side. After ensuring that all fluidic path surfaces had been wetted, the solution was incubated in the devices for at least one hour at room temperature.
3. Discarded the passivation solution by pumping it through the systems and dumping to waste. Rinsed the InnovaPrep systems thoroughly by passing 10 mL of sterile distilled water through each.

The passivation method was performed prior to the first day of testing, but was not repeated prior to the following days of testing.

2.2 Preparation of the DNA

AlburtyLab contracted with MRI to prepare a stock of Bg. This was done using the following steps:

1. An isolation streak was prepared on trypticase soy agar (TSA) from a stock culture of Bg and was incubated overnight..

2. Inoculated tryptic soy broth with a loopful of the isolated Bg colony incubated in the broth overnight while shaking.

3. Ten milliliters of the overnight culture was inoculated into 1000 mL of a sporulation medium. This sporulation culture was incubated for 48 hours with shaking.

4. Performed a purity check on the sporulation culture by performing an isolation streak onto TSA and preparing and plating 10-fold dilutions onto TSA.

5. If the purity check proved pure, the sporulation culture was centrifuged and underwent numerous wash steps prior to resuspending the Bg in a mixture of Tris HCl and lysozyme. This culture was incubated overnight with shaking.

6. The overnight lysozyme culture was centrifuged and underwent several wash steps prior to resuspending the Bg in 35 mL of Phosphate Buffered Saline (PBS).

[1] Pall Corporation, *Protein Purification and Handling,* viewed June 25, 2008, < http://www.pall.com/34696_35497.asp#25449 >.

7. The titer of the above culture was determined by preparing 10-fold serial dilutions down to the 10^{-8} dilution and plating 100 µL of the 10^{-4} through 10^{-8} dilutions in duplicate onto TSA.

The DNA was prepared from the Bg endospores using a bead-beating method described below:

1. 0.5 g of zirconium beads (diameters of 0.1, 0.5 and 1.0 mm) were placed into two separate screw cap vials with O-rings.
2. 1.2 mL of the Bg endospore preparation was placed into each of the tubes
3. Each tube was placed into the mini beadbeater and beat at the maximum RPM for 30 seconds.
4. The tubes were centrifuged at low speed for 5 minutes.
5. The supernatant fluid was removed from each tube and placed into clean microcentrifuge tubes.

2.3 Experimental Design

A flow chart of the experimental setup is shown below in Figure 2-2. Samples to be analyzed are marked with the orange numbers 1 through 7. To reduce sample analysis costs not all samples were analyzed for each test run. Samples included (1) a sub-sample of the lysed input material, (2) concentrated waste from Membrane 1, (3) a sub-sample of the permeate from Membrane 1, (4) permeate waste from Membrane 2, (5) concentrate consisting of the concentrated DNA in the first extraction of Membrane 2, and (6) a second extraction from Membrane 2. A third extraction from Membrane 2 (7) was archived. All analyses were performed in triplicate.

Feed samples and extraction fluid contained 0.025% Triton and were prepared with DNA grade water. Extraction fluid was buffered with 25 mM Tris buffer. The first membrane (Membrane 1) served as a cleanup step by removing cellular debris and other particles too large to pass through the 0.2 µm pore size. The second membrane (Membrane 2) captured the DNA on the surface while particles smaller than the 100 kD pore size passed through. The DNA was then extracted into a final concentrate of 100 µL nominal volume.

Membrane 1 contained a single 0.2 µm mixed cellulose ester membrane from Spectrum Laboratories, Inc. The effective length of this membrane is approximately 72 mm with an internal diameter of 0.6 mm. Because an air space is left at the bottom end of the fiber the length of fiber covered by liquid during operation is approximately 64 mm. This provides a utilized fiber surface area of 1.2 cm^2 with a total internal volume of 21 µL. Membrane 2 served as the concentration membrane and contained a single 100 kD polysulfone membrane (General Electric (GE) Healthcare, Inc). The effective length of this membrane is approximately 234 mm with an internal diameter of 1 mm. During operation, the length of the membrane covered by fluid is

approximately 178 mm. This provides a utilized fiber surface area of 5.6 cm^2 and a total internal volume of 190 µL. Both of these membranes were removed from standard off-the-shelf hollow fiber units and repotted into the units built by AlburtyLab.

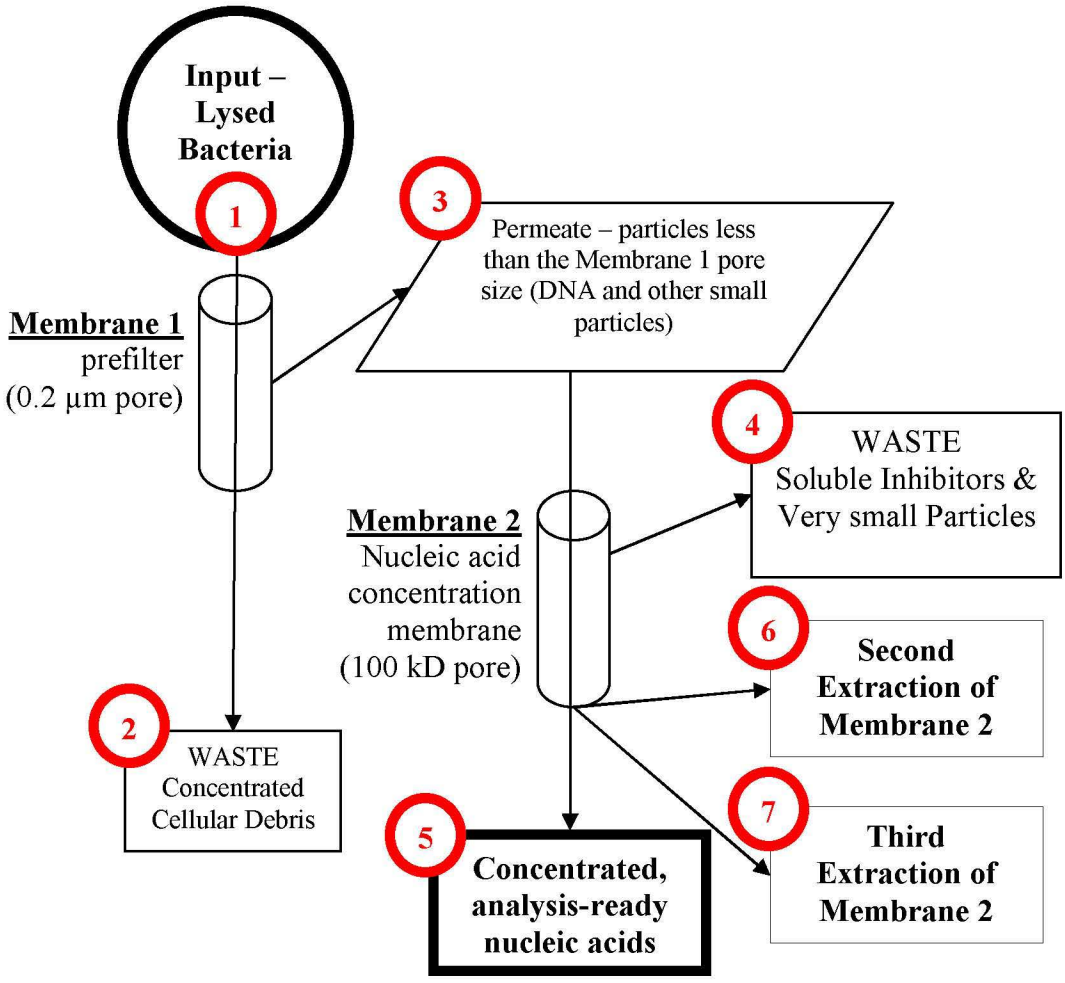

Figure 2-2. Flow Chart of the Test

Membrane 1 flux was estimated to be in the range of 350 to 550 µm/second with transmembrane pressures of 9 to 15 psi. Pressure was only applied to the feed side. The permeate side of the hollow fiber was open to atmospheric pressure. Time required for feed throughput plus extraction of the membrane ranged from approximately 3.5 minutes to 5 minutes. Some of the later runs (Run 9 and later) used a small amount of 0.025% Triton to flush the feed line and a foam flush through to the permeate side to assist in removing DNA that had been retained on the internal surfaces of the system.

Membrane 2 feed pressures were substantially higher than those used for Membrane 1. Membrane 2 was used on an Innovaprep unit with a pressure transducer feed back for controlling the feed pressure. The set point used for all test runs was 20 psi. The permeate pump was run at

full rate, so that the transmembrane pressure was probably approaching 30 psi. Feed throughput plus extraction times ranged from 5.5 minutes to 10.5 minutes. The estimated flux was 33 to 75 μm/second. Extraction fluid pressure was set to 100 psi and the extraction fluid time was set to 20 milliseconds.

The high level feed suspensions were prepared by serial diluting the DNA preparation received from MRI with 0.025% Triton to a final dilution of 1 to $5x10^2$. This provided a concentration of $3.95x10^7$ copies/mL or approximately 198 ng/mL as determined by the qPCR results. The low level feed suspensions were prepared using the same method with a final dilution of the MRI DNA preparation of 1 to $5x10^4$. Results from the qPCR analysis determined a low level feed suspension concentration of $5.13x10^5$ copies/mL or approximately 2.56 ng/mL. A second set of low level feed tests were conducted using a feed with a final dilution of 1 to $4.5x10^4$. An aliquot of the feed stock from each of the second set of low level test runs was analyzed by qPCR. The concentrations ranged from $5.55x10^5$ to $8.42x10^5$ copies/mL or approximately 2.78 to 4.21 ng/mL.

The testing procedures used are summarized below:

1. Pipet 10 mL of the feed into a tared 50 mL falcon tube and vortex. Pipet 200 uL of the feed into a tared 1.7 mL microcentrifuge tube for analysis by MRI. Record the final weights on the testing run sheet prior to loading the falcon tube sample into the feed reservoir of the cleanup InnovaPrep System.
2. Recover the Membrane 1 permeate into a tared 50 mL falcon tube and the extract of Membrane 1 into a tared 1.7 mL microcentrifuge tube. Record the final weights on the testing run sheet.
3. After vortexting the recovered Membrane 1 permeate, pipet 200 uL of the Membrane 1 permeate into a tared 1.7 mL microcentrifuge tube for analysis by MRI. Record the final weight of the microcentrifuge tube on the testing run sheet.
4. Load the remaining Membrane 1 permeate into the feed reservoir of final InnovaPrep© system.
5. Recover the Membrane 2 permeate into a tared 50 mL falcon tube and the extract of Membrane 2 into a tared 1.7 mL microcentrifuge tube. Record the final weights on the testing run sheet.
6. Perform a second extraction of the final InnovaPrep© system membrane and recover the extract into a tared 1.7 mL microcentrifuge tube. Record the final weight on the testing run sheet.
7. Perform a third extraction of the final InnovaPrep© system membrane and recover the extract into a tared 1.7 mL microcentrifuge tube. Record the final weight on the testing run sheet.
8. Perform a blank run by processing 10 mL of the 0.025% Triton solution through both InnovaPrep systems. Recover the extract of the final InnovaPrep© system into a tared 1.7 mL microcentrifuge tube. Record the final weight on the testing run sheet.
9. Start next test.

2.4 Sample Analysis

The processed samples collected during the testing were analyzed by MRI using qPCR as described below:

1. 12.5 µL of BioRad iQ Multiplex Powermix was combined with 0.5 µL of Bg RecA forward primer, 0.5 µL of Bg RecA reverse primer, 0.5 µL of Bg RecA Probe, and 6 µL of water to generate the PCR reaction mixture.
2. 5 µL of sample was added to the reaction mixture to generate a total reaction volume of 25 µL.
3. 25-µL reactions for each sample were prepared in triplicate and added to a 96-well PCR plate.
4. The standard curve was generated by preparing l0-fold serial dilutions of stock Bg DNA with known quantity and adding 5 µL of a dilution to 20 µL of PCR reaction mixture. Standard curve samples were generated in duplicate and ranged from 10^1 to 10^5 copies/reaction.
5. Thermocycling conditions were as follows:
    a. Cycle 1: (1X)
        i. Step 1: 95.0°C for 3 min
    b. Cycle 2: (45X)
        i. Step 1: 95.0°C for 15 sec
        ii. Step 2: 60.0°C for 30 sec
    c. Cycle 3: (1X)
        i. Step 1: 4.0°C hold
6. Ct values for the unknown sample quantities were compared to the standard curve (by the analysis software) to determine the copies/reaction detected in each sample.

2.5 Data Reduction and Calculations

The efficiency for passing the DNA through Membrane 1 was calculated for each high concentration run using the following formula (subscripts refer to numbers on Figure 2-2):

$$\text{Efficiency}_{\text{Membrane 1}} = \frac{C_3 \times V_3}{C_1 \times V_1} \times 100\%$$

where: C_1 = DNA concentration in Membrane 1 feed (copies/mL)
V_1 = Volume of sample processed in the InnovaPrep system (mL)
C_3 = DNA concentration in Membrane 1 permeate (copies/mL)
V_3 = Volume of permeate recovered from Membrane 1 (mL)

The efficiency of recovery of the DNA in the concentrate from Membrane 2 was calculated for each run using the following formula (subscripts refer to numbers on Figure 2-2):

$$\text{Efficiency}_{\text{Membrane 2}} = \frac{C_5 \times V_5}{C_3 \times V_3} \times 100\%$$

where: C_3 = DNA concentration in Membrane 1 permeate (copies/mL)
V_3 = Volume of permeate recovered from Membrane 1 (mL)
C_5 = DNA concentration in Membrane 2 concentrate sample (copies/mL)
V_5 = Volume of Membrane 2 concentrate sample (mL)

The total efficiency for the two membrane system will then be determined as follows:

$$\text{Overall Efficiency} = \text{Efficiency}_{\text{Membrane 1}} \times \text{Efficiency}_{\text{Membrane 2}}$$

The Dual Column InnovaPrep system concentration factor (CF) will be determined for each run using the following equation (subscripts refer to numbers on Figure 2-2):

$$CF = \frac{C_5}{C_1}$$

where: C_1 = DNA concentration in Membrane 1 feed (copies/mL)
C_5 = DNA concentration in Membrane 2 concentrate sample (copies/mL)

3. Results and Discussion

The results of the testing are presented in this section.

3.1 Titer of the Endospores

The concentration (CFU/mL) of Bg endospores in the preparation was determined to be 4.0×10^8 CFU/mL. The raw data plate counts can be seen in Table 3-1 below.

Table 3-1. Plate Counts for Endospore Titration

Dilution	Number of Colonies	
	Rep A	Rep B
-4	TNTC	TNTC
-5	330	305
-6	44	36
-7	1	3

TNTC is "too numerous to count".

The titer of the preparation was determined according to the following calculation:

$$\text{Titer} = \frac{\text{Average Plate Count}}{\text{V}} \times \text{DF}$$

where: Titer = Endospore titer in suspension (CFU/mL)
DF = Dilution factor of plated suspension
V = Volume of diluted suspension plated (0.1 mL)

$$\text{Titer} = \frac{4.0}{0.1} \times 10^6 = 4.0 \times 10^8 \text{ CFU/mL}$$

3.2 Sample Analysis

The MRI analysis results for both sets of twenty-eight samples submitted on August 13, 2008 and August 25, 2008 are presented in Appendix A.

3.3 Summary of Innovaprep Testing

A total of fifteen runs were conducted on three days of testing. Multiple changes were made to the Innovaprep protocols during testing to improve the DNA recoveries. These include the following:

Runs 1 and 2. These two low level runs were conducted on August 13, 2008. The Membrane 2 extract volume was judged to be too high for these runs. To correct for this, during all subsequent runs the following corrective actions were employed. 1) To decrease the volume of hold up liquid at the bottom of the Membrane 2, the retentate valve was closed earlier after start-up. 2) The feed pump was turned off before all the feed fluid entered the cell at the end of the run. The samples collected during this run were not submitted for analysis.

Runs 3, 7, and 8. These three low level runs were conducted on August 13, 2008. The samples collected were submitted to MRI for analysis or archive.

Runs 4, 5, and 6. These three high level runs were conducted on August 13, 2008. The samples collected were submitted to MRI for analysis or archive.

Following test runs 3 through 8, analysis of collected samples was performed at MRI. Results of these test runs were then used to adjust the InnovaPrep protocols prior to test runs 9 through 15.

Runs 9 and 10. These two low level runs were conducted on August 21, 2008. During Run 10, a leak at the top of Membrane 2 was discovered. The column was repotted on August 22, 2008. Since it was not known when the leak began, none of the samples collected during these runs were submitted for analysis.

Run 11. This low level runs was conducted on August 25, 2008. During Run 11, a leak in the permeate side of Membrane 1 was discovered. None of the samples collected during this run were submitted for analysis. Appropriate actions were taken prior to performing further runs.

Runs 12, 13, 14 and 15. These four low level runs were conducted on August 25, 2008. In order to achieve higher efficiency across Membrane 1, the following changes were made to the method employed for processing the samples with the cleanup InnovaPrep System. (1) 200 µL of 0.025% Triton X-100 in 0.2 µm sterile-filtered water was added to the feed reservoir just before the last of the feed was drawn out of the feed reservoir and into Membrane 1. It was thought that this would flush any of the sample retained on the surfaces of the feed reservoir or the tubing through to the membrane. (2) Prior to extraction of Membrane 1, three shots of extraction fluid (17 msec in length) were dispensed with the retentate valve open and a full vacuum on the permeate side of the system. This was done to flush any remaining DNA through the pores of the Membrane 1. Only a very small amount of fluid was recovered from the retentate side of the system when this was done. Changes made to the Membrane 2 processing include (1) 200 uL of 0.025% Triton in sterile water was added to the feed reservoir just before the last of the feed was drawn and (2) an attempt was made to reduce the time period that the sample was left on the membrane after the feed was processed and before the extraction took place. The samples collected during this run were submitted to MRI for analysis or archive.

3.4 Results and Discussion

The analysis results, as contained in Appendix A, combined with the sample weights were used to calculate the efficiencies and concentration factors across each column and across the entire Dual Column Innovaprep System. The detailed spreadsheet that contains all of this data can be

found in Appendix B. Table 3-2 summarizes this data. The average of the runs for each of the conditions is provided in Table 3-3.

The efficiency that DNA passed through Membrane 1 ranged from 9.0% to 14.7% for the initial low level test runs and from 74.4% to 148.4% for the high level test runs. After making some operational changes to the system four additional low level test runs were performed. The efficiencies for Membrane 1 ranged from 12.2% to 31.8%.

The efficiencies for the first extraction of Membrane 2 ranged from 12.1% to 26.5% for the initial low level test runs. High level test runs were from 25.9% to 64.1%. The second set of low level test runs ranged from 15.0% to 73.0%. Overall concentration factors for processing through Membrane 1 and 2 ranged from 1.2 to 4.1 for the initial low level test runs. The high level test runs ranged from 39.1 to 45.8 and the second set of low level test runs ranged from 3.8 to 9.0.

Table 3-2. Summary of InnovaPrep DNA Testing

	Low Level Runs			High Level Runs			Repeat of Low Level Runs			
	Run 3	Run 7	Run 8	Run 4	Run 5	Run 6	Run 12	Run 13	Run 14	Run 15
Feed										
qPCR Result, copies/mL	5.13E+05	5.13E+05	5.13E+05	3.95E+07	3.95E+07	3.95E+07	6.86E+05	8.42E+05	7.82E+05	5.55E+05
Feed Weight, g	9.95	9.79	9.97	9.80	9.92	9.87	9.60	9.66	9.55	9.59
Number Copies Fed	5.10E+06	5.02E+06	5.11E+06	3.88E+08	3.92E+08	3.90E+08	6.59E+06	8.14E+06	7.47E+06	5.32E+06
Membrane 1 Permeate/Membrane 2 Feed										
qPCR Result, copies/mL	6.04E+04	7.53E+04	4.70E+04	6.00E+07	3.33E+07	2.97E+07	2.07E+05	1.73E+05	1.14E+05	6.98E+04
Permeate Weight, g	9.83	9.79	9.77	9.59	9.77	9.77	10.13	9.81	9.90	9.32
Membrane 1, % Efficiency	11.6%	14.7%	9.0%	148.4%	82.9%	74.4%	31.8%	20.8%	15.1%	12.2%
Number Copies Fed	5.82E+05	7.22E+05	4.50E+05	5.64E+08	3.19E+08	2.85E+08	2.06E+06	1.66E+06	1.12E+06	6.37E+05
Membrane 2--Extract 1										
qPCR Result, copies/mL	9.13E+05	2.08E+06	6.23E+05	1.81E+09	1.55E+09	1.70E+09	6.09E+06	6.04E+06	7.03E+06	2.13E+06
Sample Weight, g	0.086	0.092	0.087	0.081	0.114	0.107	0.051	0.135	0.116	0.108
Number Copies	7.89E+04	1.91E+05	5.45E+04	1.46E+08	1.75E+08	1.82E+08	3.09E+05	8.12E+05	8.14E+05	2.30E+05
Membrane 2, % Efficiency	13.6%	26.5%	12.1%	25.9%	55.1%	64.1%	15.0%	48.9%	73.0%	36.1%
Total, % Efficiency	1.5%	3.8%	1.1%	37.6%	44.7%	46.7%	4.7%	10.0%	10.9%	4.3%
Membrane 2 Concentration Factor	15.1	27.6	13.3	30.1	46.5	57.3	29.4	35.0	61.8	30.5
Overall Concentration Factor	1.8	4.1	1.2	45.8	39.1	43.1	8.9	7.2	9.0	3.8
Membrane 2--Extracts 1 & 2 together										
qPCR Result, copies/mL	2.47E+05	1.12E+06	1.98E+04	1.28E+09	3.83E+08	3.60E+08	2.55E+06	1.07E+06	1.44E+06	5.63E+05
Sample Weight, g	0.146	0.111	0.103	0.103	0.132	0.108	0.132	0.109	0.125	0.146
Membrane 2, % Efficiency	19.7%	43.6%	12.6%	49.4%	70.9%	77.7%	31.4%	55.9%	89.2%	49.0%
Total % Efficiency	2.3%	6.3%	1.1%	71.8%	57.7%	56.6%	9.8%	11.4%	13.3%	5.9%
Membrane 2 Concentration Factor	8.2	20.7	6.3	25.2	27.7	34.7	17.1	22.1	36.4	17.6
Overall Concentration Factor	1.0	3.0	0.6	38.3	23.3	26.0	5.2	4.5	5.3	2.2
Membrane 2--Extracts 1, 2, & 3 together										
qPCR Result, copies/mL	--	--	--	--	--	--	--	1.62E+05	4.55E+05	2.57E+05
Sample Weight, g	--	--	--	--	--	--	--	0.101	0.104	0.116
Membrane 2, % Efficiency	--	--	--	--	--	--	--	56.9%	93.4%	53.7%
Total % Efficiency	--	--	--	--	--	--	--	11.6%	13.9%	6.4%
Membrane 2 Concentration Factor	--	--	--	--	--	--	--	15.9	26.6	13.2
Overall Concentration Factor	--	--	--	--	--	--	--	3.3	3.9	1.7
Blank Extract Performed after Run										
qPCR Result, copies/mL	--	--	--	6.79E+07	--	--	3.51E+05	6.57E+05	9.43E+05	2.94E+05
% of Last Run	--	--	--	2.1%	--	--	0.6%	0.9%	1.3%	0.8%

Table 3-3. Average Efficiencies and Concentration Factors for InnovaPrep DNA Testing

	Low Level Runs		High Level Runs		Repeat of Low Level Runs	
	Average	SD	Average	SD	Average	SD
Membrane 1, % Efficiency	11.8%	2.9%	101.9%	40.5%	20.0%	8.7%
Membrane 2--Extract 1						
Membrane 2, % Efficiency	17.4%	7.9%	48.3%	20.0%	43.3%	24.3%
Total % Efficiency	2.1%	1.5%	43.0%	4.8%	7.5%	3.5%
Membrane 2 Concentration Factor	18.7	7.8	44.6	13.7	39.2	15.3
Overall Concentration Factor	2.4	1.5	42.6	3.3	7.2	2.4
Membrane 2--Extracts 1 & 2 together						
Membrane 2, % Efficiency	25.3%	16.3%	66.0%	14.8%	56.4%	24.2%
Total % Efficiency	3.2%	2.7%	62.0%	8.5%	10.1%	3.2%
Membrane 2 Concentration Factor	11.7	7.8	29.2	4.9	23.3	9.0
Overall Concentration Factor	1.5	1.3	29.2	8.0	4.3	1.4
Membrane 2--Extracts 1, 2, & 3 together						
Membrane 2, % Efficiency	--	--	--	--	68.0%	22.1%
Total % Efficiency	--	--	--	--	10.7%	3.9%
Membrane 2 Concentration Factor	--	--	--	--	18.6	7.1
Overall Concentration Factor	--	--	--	--	2.9	1.1

Since performing the test runs described in this report, additional paper study and laboratory work were performed outside this program by AlburtyLab. The goal of this was to acquire further information that may assist in improving the observed efficiencies. Journal articles were found that pertain to the passage of DNA and other particles through hollow fiber membranes. The majority of the research sited in these articles was performed with the goal of improving the efficiency with which DNA passes through 0.2 μm and smaller pore size membranes during membrane sterilization of pharmaceutical production of plasmid DNA. This is a very recent research area and it is currently focused on production of large quantities of high purity plasmid DNA for gene therapy and DNA-based vaccines. The research that has been performed is applicable to the InnovaPrep system; however the majority of this data is for DNA concentrations that are one or more orders of magnitude greater than those used for this study. It is possible to extrapolate from some of this data back to the concentrations used for this study, but it is not entirely clear whether this is meaningful for all of the parameters discussed below.

Multiple authors have investigated the parameters that control whether DNA passes through or is retained by a membrane. The parameters that have been observed to have the greatest effect are membrane pore size, size of DNA, total concentration of particles in solution, solution conductivity or salt concentration, pH, and flux rate or trans-membrane pressure. For a given pore size it is possible to significantly affect the retention or passage of DNA by controlling the pH, salt concentration, or trans-membrane pressure. In general, an increase in any of these three parameters causes an increase in passage of DNA. An overview of research that has been performed in this area is shown below in Table 3-4.

DNA was passed through Membrane 1 efficiently at the high concentrations but not at the lower concentrations. From review of the data presented in the referenced articles it also appears that Membrane 1 should have passed the DNA with high efficiencies. Buffering the pH of the feed solution and adding a salt should reduce charge interactions that may have reduced the efficiency of Membrane 1 and Membrane 2. The addition of the salt will act to increase passage of DNA through Membrane 2. Therefore it will be necessary to reduce the pore size and reduce the transmembrane pressure for Membrane 2 in order to reduce passage of DNA.

Table 3-4. Summary of DNA Fractionation Research

Researcher	DNA type	Membrane type	Membrane Pore Size	Flux (μm/s)	Transmembrane Pressure psi	Salt Conc. (mM)	pH	DNA Conc. (ng/ml)
Latulippe et al.[1,2,3]	3 kb circular plasmid	Flat/stirred cell–regen. Cellulose, PVDF (0.22 μm)	100 kD, 300 kD, 1000 kD, 0.22 μm	100 kD–18 to 105 300 kD–20 to 125 1000 kD–4 to 80 0.22 μm–6 to 135	100 kD–0.5 to 5.8 300 kD–0.5 to 4.1 1000 kD–0.5 to 1.7 0.22 μm–not report.	1, 10, 40, 150 NaCl	7.7	250; 750; 2,500
Hirasaki et al.[4]	3 kb circular plasmid, 46 kb & 154 kb DS lin.	BMM Dead-end hollow fiber regen. cellulose	15 nm (~200 kD), 35 nm (~475 kD)	-	0.5, 1.0, 1.9, 3.9	Tris/ EDTA	7.5	2,150; 5,300; 11,250; 15,000
Higuchi et al.[5]	2kb to 23 kb SS & DS lin.	BMM Dead-end hollow fiber (30cm^2) regen. cellulose	15 nm (~200 kD)	1.1 to 2.2	3.9	Tris/ EDTA	7.4 to 8.0	10,000; 25,000; 50,000; 75,000; 100,000
Arkhangelsky et al.[6]	9.5 kb circular plasmid	Flat/stirred cell cellulose acetate, PES	20 kD	-	7.25, 14.5, 29.0, 43.5, 58.0, 72.5	10, 50, 100 NaCl	3,4,5,6,7,8,9, 10, 11	330
Kong et al.[7]	6, 20, 29, 33, 72, 116 kb Circular plasmid	Flat PVDF (0.22 μm), PES (0.2 μm)	0.22 μm, 0.2 μm	17, 33, 133, 383		Tris/ EDTA, 150 NaCl	8.0	25,000 to 100,000
InnovaPrep – Stage 1	Total-bead beat Bg	Dead-end hollow fiber mixed cellulose ester	0.2 μm	300 to 500	9 to 15	None – 0.025% Triton	No buffer	2.56 to 198
InnovaPrep – Stage 2	Total-bead beat Bg	Dead-end hollow fiber polysulfone	100 kD	33 to 75	25 to 30	None – 0.025% Triton	No buffer	2.56 to 198

3.5 Data Archival

Laboratory samples (biological samples) will be temporarily archived at MRI. All supporting data is recorded on the testing log sheets and in AlburtyLab Lab Notebook #2, pages 185-191. Copies of all supporting data are archived in the project file and will be available on request for at least 3 years.

4. Conclusions and Recommendations for Future Testing

Advanced systems that provide automated purification and concentration of DNA from aqueous samples are virtually non-existent. The InnovaPrep technology holds significant promise for automated preparation of DNA for analysis by PCR or other methods. By passing DNA through a large pore membrane, cellular debris and other large particles can be removed from the sample. The sample can then be captured onto a second smaller pore size membrane where the DNA is captured and the sample fluid is removed and replaced with a small volume of clean analysis compatible buffer.

Efficiencies for DNA processing were lower than may be deemed acceptable for implementation into some current biosampling and detection systems, however concentration factors for all ten test runs were above 1x and thereby provide a net increase in sample concentration to the detector. After several operation changes were made an additional three low level test runs were performed. These test runs provided an average concentration factor of 7.2x with a standard deviation of 2.4.

AlburtyLab believes that significant improvement in the efficiency of the InnovaPrep system can be made with minimal operational changes to the system. Seven key changes have been identified that will provide significant improvement to the system.

1. Feed should be 25 mM Tris, 0.15 M NaCl, pH 7.2, 0.05% Triton X-100, and 1 mM EDTA or similar. Salt concentration should be kept above about 50 mM.
2. Replace existing Norprene® peristaltic tubing sets with platinum cured silicon tubing.
3. In order to reduce pulsing and formation of bubbles and foam in the fluid line use the smallest peristaltic tubing size that will deliver the required flow rate and use improved peristaltic pumps.
4. Use the shortest practical tubing length and if possible, flush tubing with foam or liquid after each step to reduce losses.
5. For the stage one membrane use 0.65 micrometer, 1 mm ID, polysulfone membrane with approximately 16 cm^2 surface area. Operate the membrane at pressures up to approximately 2 psi transmembrane pressure. At 2 psi a flux of approximately 37 ml/min is possible. Higher pressures are acceptable and should not significantly reduce efficiencies but may increase the rate of membrane flux decline.
6. Efforts should be made in the system design to keep all fluid path surfaces completely covered with fluid whenever possible. This is important to keep DNA from contacting dry or partially dry surfaces and binding to those surfaces. It is especially difficult to keep the outer surface of Membrane 1 covered with fluid. As an alternative to keep the surfaces covered in fluid it may be possible to flush the surfaces with clean feed solution or extraction foam.
7. For the stage two membrane use a 50 kD pore size, 0.5 mm id, polysulfone membrane with approximately 26 cm^2 surface area. Operate the membrane at a maximum

transmembrane pressure of approximately 6 psi. A flux of approximately 2 mL/min is possible at 6 psi.

As an initial step AlburtyLab plans to implement these changes and retest the system. We plan to use a Picogreen fluorescent dye labeling method to determine the concentration of DNA in the feed, permeate, and concentrate. As part of this testing we plan to evaluate the ability of the system to remove soluble and insoluble materials. A final test will involve retesting the system with low levels of DNA with analysis by qPCR.

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Appendix A

References

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Appendix B

MRI Sample Analysis Results

Table B-1. Results for Samples 1 through 28 for RFQ-02-2008

MRI Sample Number	Sample Name	Sample Description	Copies/5µL			Average Copies/5µL	Average Copies/mL	Std Dev Copies/mL
			Rep. A	Rep. B	Rep. C			
1	AL-02-2008-01	Spike Material	7.07E+07	7.92E+07	6.69E+07	7.23E+07	1.45E+10	6.30E+06
2	AL-02-2008-02	Low Level Feed Run 1	2.53E+03	2.59E+03	2.57E+03	2.56E+03	5.13E+05	3.06E+01
3	AL-02-2008-42	Membrane 1 Permeate Run 7	3.99E+02	3.46E+02	3.84E+02	3.76E+02	7.53E+04	2.73E+01
4	AL-02-2008-44	Membrane 2 Extract Run 7	1.33E+04	8.37E+03	9.54E+03	1.04E+04	2.08E+06	2.58E+03
5	AL-02-2008-45	Membrane 2 Extract 2 Run 7	4.72E+03	5.12E+03	6.96E+03	5.60E+03	1.12E+06	1.19E+03
6	AL-02-2008-49	Membrane 1 Permeate Run 8	2.66E+02	2.76E+02	1.63E+02	2.35E+02	4.70E+04	6.26E+01
7	AL-02-2008-51	Membrane 2 Extract Run 8	3.82E+03	3.16E+03	2.37E+03	3.12E+03	6.23E+05	7.26E+02
8	AL-02-2008-52	Membrane 2 Extract 2 Run 8	1.18E+02	1.31E+02	4.85E+01	9.92E+01	1.98E+04	4.44E+01
9	AL-02-2008-16	Membrane 1 Permeate Run 3	2.27E+02	2.90E+02	3.89E+02	3.02E+02	6.04E+04	8.17E+01
10	AL-02-2008-18	Membrane 2 Extract Run 3	4.98E+03	4.75E+03	3.97E+03	4.57E+03	9.13E+05	5.29E+02
11	AL-02-2008-19	Membrane 2 Extract 2 Run 3	1.13E+03	1.23E+03	1.34E+03	1.23E+03	2.47E+05	1.05E+02
12	AL-02-2008-21	High Level Feed Run 4	2.23E+05	2.07E+05	1.63E+05	1.98E+05	3.95E+07	3.11E+04
13	AL-02-2008-22	Membrane 1 Waste Run 4	1.02E+05	1.11E+05	1.51E+05	1.21E+05	2.43E+07	2.61E+04
14	AL-02-2008-23	Membrane 1 Permeate Run 4	3.39E+05	3.11E+05	2.50E+05	3.00E+05	6.00E+07	4.55E+04
15	AL-02-2008-24	Membrane 2 Waste Run 4	5.10E+01	2.47E+01	1.12E+01	2.90E+01	5.79E+03	2.02E+01
16	AL-02-2008-25	Membrane 2 Extract Run 4	1.00E+07	8.50E+06	8.63E+06	9.04E+06	1.81E+09	8.31E+05
17	AL-02-2008-26	Membrane 2 Extract 2 Run 4	6.33E+06	6.95E+06	5.96E+06	6.41E+06	1.28E+09	5.00E+05
18	AL-02-2008-28	Membrane 1 Waste Run 5	1.17E+06	1.08E+06	1.02E+06	1.09E+06	2.18E+08	7.55E+04
19	AL-02-2008-29	Membrane 1 Permeate Run 5	2.16E+05	1.63E+05	1.20E+05	1.66E+05	3.33E+07	4.81E+04
20	AL-02-2008-30	Membrane 2 Waste Run 5	1.58E+01	2.33E+01	2.55E+01	2.15E+01	4.31E+03	5.09E+00
21	AL-02-2008-31	Membrane 2 Extract Run 5	6.77E+06	8.04E+06	8.38E+06	7.73E+06	1.55E+09	8.49E+05
22	AL-02-2008-32	Membrane 3 Extract 2 Run 5	1.99E+06	2.02E+06	1.73E+06	1.91E+06	3.83E+08	1.59E+05
23	AL-02-2008-34	Blank Extraction of Membrane 2 Run 5	2.95E+05	4.03E+05	3.20E+05	3.39E+05	6.79E+07	5.65E+04
24	AL-02-2008-35	Membrane 1 Waste Run 6	1.00E+06	7.69E+05	7.96E+05	8.55E+05	1.71E+08	1.26E+05
25	AL-02-2008-36	Membrane 1 Permeate Run 6	NA	1.35E+05	1.62E+05	1.49E+05	2.97E+07	1.91E+04
26	AL-02-2008-37	Membrane 2 Waste Run 6	4.94E+01	5.53E+01	5.15E+01	5.21E+01	1.04E+04	2.99E+00
27	AL-02-2008-38	Membrane 2 Extract Run 6	9.11E+06	8.56E+06	7.86E+06	8.51E+06	1.70E+09	6.26E+05
28	AL-02-2008-39	Membrane 2 Extract 2 Run 6	2.25E+06	1.70E+06	1.45E+06	1.80E+06	3.60E+08	4.09E+05

Table B-2. Results for Samples 1 through 28 for RFQ-02-2008

MRI Sample Number	Sample Name	Sample Description	Copies/5µL			Average Copies/5µL	Average Copies/mL	Std Dev Copies/mL
			Rep. A	Rep. B	Rep. C			
1	AL-02-2008-149	Pre-Run Blank Extract, Run 15	1.51E+03	1.24E+03	1.66E+03	1.47E+03	2.94E+05	2.13E+02
2	AL-02-2008-150	Membrane 1 Feed, Run 15	2.09E+03	3.08E+03	3.16E+03	2.78E+03	5.55E+05	5.96E+02
3	AL-02-2008-151	Membrane 1 Waste, Run 15	1.89E+04	1.63E+04	1.51E+04	1.68E+04	3.35E+06	1.94E+03
4	AL-02-2008-152	Membrane 2 Feed, Run 15	3.30E+02	3.76E+02	3.41E+02	3.49E+02	6.98E+04	2.40E+01
5	AL-02-2008-154	Membrane 2 Extract, Run 15	9.34E+03	1.11E+04	1.15E+04	1.06E+04	2.13E+06	1.15E+03
6	AL-02-2008-155	Membrane 2 Extract 2, Run 15	2.80E+03	2.81E+03	2.83E+03	2.81E+03	5.63E+05	1.53E+01
7	AL-02-2008-156	Membrane 2 Extract 3, Run 15	1.17E+03	1.35E+03	1.33E+03	1.28E+03	2.57E+05	9.87E+01
8	AL-02-2008-125	Pre-Run Blank Extract, Run 12	1.76E+03	1.96E+03	1.54E+03	1.75E+03	3.51E+05	2.10E+02
9	AL-02-2008-126	Membrane 1 Feed, Run 12	2.82E+03	3.55E+03	3.92E+03	3.43E+03	6.86E+05	5.60E+02
10	AL-02-2008-127	Membrane 1 Waste, Run 12	6.07E+03	6.05E+03	7.13E+03	6.42E+03	1.28E+06	6.18E+02
11	AL-02-2008-128	Membrane 2 Feed, Run 12	1.07E+03	1.12E+03	9.13E+02	1.03E+03	2.07E+05	1.08E+02
12	AL-02-2008-130	Membrane 2 Extract, Run 12	3.45E+04	3.34E+04	2.34E+04	3.04E+04	6.09E+06	6.12E+03
13	AL-02-2008-131	Membrane 2 Extract 2, Run 12	1.32E+04	1.38E+04	1.13E+04	1.28E+04	2.55E+06	1.31E+03
14	AL-02-2008-133	Pre-Run Blank Extract, Run 13	2.89E+03	3.57E+03	3.40E+03	3.29E+03	6.57E+05	3.54E+02
15	AL-02-2008-134	Membrane 1 Feed, Run 13	4.96E+03	4.24E+03	3.43E+03	4.21E+03	8.42E+05	7.65E+02
16	AL-02-2008-136	Membrane 2 Feed, Run 13	9.22E+02	9.23E+02	7.46E+02	8.64E+02	1.73E+05	1.02E+02
17	AL-02-2008-135	Membrane 1 Waste, Run 13	1.16E+04	1.48E+04	1.53E+04	1.39E+04	2.78E+06	2.01E+03
18	AL-02-2008-138	Membrane 2 Extract, Run 13	2.69E+04	3.37E+04	3.00E+04	3.02E+04	6.04E+06	3.40E+03
19	AL-02-2008-139	Membrane 2 Extract 2, Run 13	5.73E+03	5.70E+03	4.60E+03	5.34E+03	1.07E+06	6.44E+02
20	AL-02-2008-140	Membrane 2 Extract 3, Run 13	8.79E+02	8.03E+02	7.45E+02	8.09E+02	1.62E+05	6.72E+01
21	AL-02-2008-141	Pre-Run Blank Extract, Run 14	4.05E+03	5.06E+03	5.04E+03	4.72E+03	9.43E+05	5.77E+02
22	AL-02-2008-142	Membrane 1 Feed, Run 14	4.04E+03	3.95E+03	3.74E+03	3.91E+03	7.82E+05	1.54E+02
23	AL-02-2008-143	Membrane 1 Waste, Run 14	2.71E+04	2.31E+04	2.32E+04	2.45E+04	4.89E+06	2.28E+03
24	AL-02-2008-144	Membrane 2 Feed, Run 14	6.24E+02	5.35E+02	5.47E+02	5.69E+02	1.14E+05	4.83E+01
25	AL-02-2008-145	Membrane 2 Waste, Run 14	1.40E+01	No Amp	1.07E+01	1.24E+01	2.47E+03	2.33E+00
26	AL-02-2008-146	Membrane 2 Extract, Run 14	3.56E+04	3.08E+04	3.91E+04	3.52E+04	7.03E+06	4.17E+03
27	AL-02-2008-147	Membrane 2 Extract 2, Run 14	7.59E+03	7.12E+03	6.95E+03	7.22E+03	1.44E+06	3.32E+02
28	AL-02-2008-148	Membrane 2 Extract 3, Run 14	2.24E+03	2.48E+03	2.11E+03	2.28E+03	4.55E+05	1.88E+02

Appendix C

Data Reduction Worksheets

Table C-1. Data Reduction for the DNA InnovaPrep Concentration Project (LWI 2008-01)

	Low Level Runs			Line ID	
	Run 3	Run 7	Run 8		Source/Formula
Spike Material	AL-02-2008-01				
qPCR Result, copies/mL	1.45E+10	--	--		
Feed	AL-02-2008-02				
qPCR Result, copies/mL	5.13E+05	5.13E+05	5.13E+05	a	From MRI Results Sheet
Feed & Vial Gross Weight, g	17.4289	17.1298	17.5276	b	From Test Run Sheet
Vial Tare Weight, g	7.4743	7.3420	7.5626	c	From Test Run Sheet
Feed Weight, g	9.9546	9.7878	9.9650	d	d = b - c
Sample & Vial Gross Weight, g	2.0342	--	--	e	From Test Run Sheet
Vial Tare Weight, g	1.0369	--	--	f	From Test Run Sheet
Sample Weight, g	0.9973	--	--	g	g = e - f
Number Copies Fed	5.10E+06	5.02E+06	5.11E+06	h	h = a * g
Membrane 1 Waste	AL-02-2008-15	AL-02-2008-41	AL-02-2008-48		
qPCR Result, copies/mL	--	--	--	i	From MRI Results Sheet
Sample & Vial Gross Weight, g	1.1679	1.1616	1.1715	j	From Test Run Sheet
Vial Tare Weight, g	1.0606	1.0549	1.0412	k	From Test Run Sheet
Sample Weight, g	0.1073	0.1067	0.1303	l	d = b - c
Number Copies	--	--	--	m	m = l * i
% Lost to Membrane 1 Waste	--	--	--	n	n = m / h x 100%
Membrane 1 Permeate/Membrane 2 Feed	AL-02-2008-16	AL-02-2008-42	AL-02-2008-49		
qPCR Result, copies/mL	6.04E+04	7.53E+04	4.70E+04	o	From MRI Results Sheet
Permeate & Vial Gross Weight, g	17.1964	NA	16.9147	p	From Test Run Sheet
Vial Tare Weight, g	7.3617	7.3355	7.1480	q	From Test Run Sheet
Permeate Weight, g	9.8347	9.7878	9.7667	r	r = q - p
Sample & Vial Gross Weight, g	1.2554	1.2721	1.2282	s	From Test Run Sheet
Vial Tare Weight, g	1.0600	1.0791	1.0360	t	From Test Run Sheet
Sample Weight, g	0.1954	0.1930	0.1922	u	u = s - t
Membrane 2 Feed Weight, g	9.6393	9.5948	9.5745	v	v = r - u
Number Copies in Permeate	5.94E+05	7.37E+05	4.59E+05	w	w = o * r
Membrane 1 % Efficiency	11.6%	14.7%	9.0%	x	x = w / h
Number Copies Fed	5.82E+05	7.22E+05	4.50E+05	y	y = o * v
Membrane 2 Waste	AL-02-2008-17	AL-02-2008-43	AL-02-2008-50		
qPCR Result, copies/mL	--	--	--	z	From MRI Results Sheet
Sample & Vial Gross Weight, g	16.2721	15.1042	16.8054	aa	From Test Run Sheet
Vial Tare Weight, g	7.1645	7.4994	7.2441	ab	From Test Run Sheet
Sample Weight, g	9.1076	7.6048	9.5613	ac	ac = aa - ab
Number Copies	--	--	--	ad	ad = ac * z

Table C-1. Data Reduction for the DNA InnovaPrep Concentration Project (LWI 2008-01)

	Low Level Runs			Line ID	
	Run 3	Run 7	Run 8		Source/Formula
- % Total Lost to Membrane 2 Waste	--	--	--	ae	ae = ad / y x 100%
Membrane 2 Extract 1	AL-02-2008-18	AL-02-2008-44	AL-02-2008-51		
- qPCR Result, copies/mL	9.13E+05	2.08E+06	6.23E+05	af	From MRI Results Sheet
- Sample & Vial Gross Weight, g	1.1491	1.1799	1.1257	ag	From Test Run Sheet
- Vial Tare Weight, g	1.0627	1.0880	1.0382	ah	From Test Run Sheet
- Sample Weight, g	0.0864	0.0919	0.0875	ai	ai = ag - ah
- Number Copies	7.89E+04	1.91E+05	5.45E+04	aj	aj = ai * af
- Membrane 2 % Efficiency	13.6%	26.5%	12.1%	ak	ak = aj / y * 100%
- Total % Efficiency	1.5%	3.8%	1.1%	al	al = aj / h * 100%
- Membrane 2 Concentration Factor	15.1	27.6	13.3	am	am = af / o
- Overall Concentration Factor	1.8	4.1	1.2	an	am = af / a
Membrane 2 Extract 2	AL-02-2008-19	AL-02-2008-45	AL-02-2008-52		
- qPCR Result, copies/mL	2.47E+05	1.12E+06	1.98E+04	ao	From MRI Results Sheet
- Sample & Vial Gross Weight, g	1.2112	1.1596	1.1331	ap	From Test Run Sheet
- Vial Tare Weight, g	1.0650	1.0490	1.0301	aq	From Test Run Sheet
- Sample Weight, g	0.1462	0.1106	0.1030	ar	ar = ap - aq
- Number Copies	3.61E+04	1.24E+05	2.04E+03	as	as = ar * ao
- Membrane 2 % Efficiency	6.2%	17.2%	0.5%	at	at = as / y * 100%
Membrane 2 Extract 1 & 2 together					
- Total Sample Weight, g	0.2326	0.2025	0.1905	be	be = ar + ai
- Membrane 2 % Efficiency	19.7%	43.6%	12.6%	au	au = (as + aj) / y * 100%
- Total % Efficiency	2.3%	6.3%	1.1%	bf	bf = (as + aj) / h
- Membrane 2 Concentration Factor	8.2	20.7	6.3	bg	bg = ((as + aj) / bl) / o
- Overall Concentration Factor	1.0	3.0	0.6	bh	bh = ((as + aj) / bl) / a
Membrane 2 Extract 3	AL-02-2008-20	AL-02-2008-46	AL-02-2008-53		
- qPCR Result, copies/mL	--	--	--	bi	From MRI Results Sheet
- Sample & Vial Gross Weight, g	1.1908	1.1336	1.1303	av	From Test Run Sheet
- Vial Tare Weight, g	1.0664	1.0446	1.0233	aw	From Test Run Sheet
- Sample Weight, g	0.1244	0.0890	0.1070	ax	ax = av - aw
- Number Copies	--	--	--	bj	bh = bg * ax
- Membrane 3 % Efficiency	--	--	--	bk	bi = bh / y * 100%
Membrane 2 Extract 1, 2, & 3 together					
- Total Sample Weight, g				bl	bl = ax + ar + ai
- Membrane 2 % Efficiency				bm	bm = (bj + as + aj) / y * 100%
- Total % Efficiency	--	--	--	bn	bl = (bj + as + aj) / h * 100%
- Membrane 2 Concentration Factor				bo	bo = ((bj + as + aj) / bl) / o
- Overall Concentration Factor	--	--	--	bp	bp = ((bj + as + aj) / bl) / a

Table C-1. Data Reduction for the DNA InnovaPrep Concentration Project (LWI 2008-01)

	Low Level Runs			Line ID	
	Run 3	Run 7	Run 8		Source/Formula
Blank Extract Performed after Run	No Number	No Number	No Number		
qPCR Result, copies/mL	--	--	--	ay	From MRI Results Sheet
Sample & Vial Gross Weight, g	1.1994	1.1879	1.4024	az	From Test Run Sheet
Vial Tare Weight, g	1.0947	1.0641	1.0866	ba	From Test Run Sheet
Sample Weight, g	0.1047	0.1238	0.3158	bb	bb = az - ba
Number Copies	--	--	--	bc	bc = bb * ay
% of Last Run				bd	bd = bc / h * 100%

Table C-2. Data Reduction for the DNA InnovaPrep Concentration Project (LWI 2008-01)

	High Level Runs			Line ID	
	Run 4	Run 5	Run 6		Source/Formula
Feed	AL-02-2008-21				
qPCR Result, copies/mL	3.95E+07	3.95E+07	3.95E+07	a	From MRI Results Sheet
Feed & Vial Gross Weight, g	16.9969	17.0999	17.0689	b	From Test Run Sheet
Vial Tare Weight, g	7.1945	7.1808	7.1970	c	From Test Run Sheet
Feed Weight, g	9.8024	9.9191	9.8719	d	d = b - c
Sample & Vial Gross Weight, g	1.2271	--	--	e	From Test Run Sheet
Vial Tare Weight, g	1.0298	--	--	f	From Test Run Sheet
Sample Weight, g	0.1973	--	--	g	g = e - f
Number Copies Fed	3.88E+08	3.92E+08	3.90E+08	h	h = a * g
Membrane 1 Waste	AL-02-2008-22	AL-02-2008-28	AL-02-2008-35		
qPCR Result, copies/mL	2.43E+07	2.18E+08	1.71E+08	i	From MRI Results Sheet
Sample & Vial Gross Weight, g	1.1673	1.1833	1.1681	j	From Test Run Sheet
Vial Tare Weight, g	1.0579	1.0685	1.0381	k	From Test Run Sheet
Sample Weight, g	0.1094	0.1148	0.1300	l	d = b - c
Number Copies	2.65E+06	2.50E+07	2.22E+07	m	m = l * i
% Lost to Membrane 1 Waste	0.7%	6.4%	5.7%	n	n = m / h x 100%
Membrane 1 Permeate/Membrane 2 Feed	AL-02-2008-23	AL-02-2008-29	AL-02-2008-36		
qPCR Result, copies/mL	6.00E+07	3.33E+07	2.97E+07	o	From MRI Results Sheet
Permeate & Vial Gross Weight, g	16.8036	16.9780	17.3113	p	From Test Run Sheet
Vial Tare Weight, g	7.2162	7.2067	7.5383	q	From Test Run Sheet
Permeate Weight, g	9.5874	9.7713	9.7730	r	r = q - p
Sample & Vial Gross Weight, g	1.2473	1.2396	1.2626	s	From Test Run Sheet
Vial Tare Weight, g	1.0550	1.0484	1.0702	t	From Test Run Sheet
Sample Weight, g	0.1923	0.1912	0.1924	u	u = s - t
Membrane 2 Feed Weight, g	9.3951	9.5801	9.5806	v	v = r - u
Number Copies in Permeate	5.75E+08	3.25E+08	2.90E+08	w	w = o * r
Membrane 1 % Efficiency	148.4%	82.9%	74.4%	x	x = w / h
Number Copies Fed	5.64E+08	3.19E+08	2.85E+08	y	y = o * v
Membrane 2 Waste	AL-02-2008-24	AL-02-2008-30	AL-02-2008-37		
qPCR Result, copies/mL	5.79E+03	4.31E+03	1.04E+04	z	From MRI Results Sheet
Sample & Vial Gross Weight, g	16.4345	16.6083	17.1413	aa	From Test Run Sheet
Vial Tare Weight, g	7.1986	7.5144	7.5365	ab	From Test Run Sheet
Sample Weight, g	9.2359	9.0939	9.6048	ac	ac = aa - ab
Number Copies	5.35E+04	3.92E+04	1.00E+05	ad	ad = ac * z
% Total Lost to Membrane 2 Waste	0.01%	0.01%	0.04%	ae	ae = ad / y x 100%

Table C-2. Data Reduction for the DNA InnovaPrep Concentration Project (LWI 2008-01)

	High Level Runs			Line ID	
	Run 4	Run 5	Run 6		Source/Formula
Membrane 2 Extract 1	AL-02-2008-25	AL-02-2008-31	AL-02-2008-38		
qPCR Result, copies/mL	1.81E+09	1.55E+09	1.70E+09	af	From MRI Results Sheet
Sample & Vial Gross Weight, g	1.1095	1.1582	1.1732	ag	From Test Run Sheet
Vial Tare Weight, g	1.0289	1.0447	1.0661	ah	From Test Run Sheet
Sample Weight, g	0.0806	0.1135	0.1071	ai	ai = ag - ah
Number Copies	1.46E+08	1.75E+08	1.82E+08	aj	aj = ai * af
Membrane 2 % Efficiency	25.9%	55.1%	64.1%	ak	ak = aj / y * 100%
Total % Efficiency	37.6%	44.7%	46.7%	al	al = aj / h * 100%
Membrane 2 Concentration Factor	30.1	46.5	57.3	am	am = af / o
Overall Concentration Factor	45.8	39.1	43.1	an	am = af / a
Membrane 2 Extract 2	AL-02-2008-26	AL-02-2008-32	AL-02-2008-39		
qPCR Result, copies/mL	1.28E+09	3.83E+08	3.60E+08	ao	From MRI Results Sheet
Sample & Vial Gross Weight, g	1.1488	1.2031	1.1721	ap	From Test Run Sheet
Vial Tare Weight, g	1.0454	1.0708	1.0646	aq	From Test Run Sheet
Sample Weight, g	0.1034	0.1323	0.1075	ar	ar = ap - aq
Number Copies	1.33E+08	5.06E+07	3.87E+07	as	as = ar * ao
Membrane 2 % Efficiency	23.5%	15.9%	13.6%	at	at = as / y * 100%
Membrane 2 Extract 1 & 2 together					
Total Sample Weight, g	0.1840	0.2458	0.2146	be	be = ar + ai
Membrane 2 % Efficiency	49.4%	70.9%	77.7%	au	au = (as + aj) / y * 100%
Total % Efficiency	71.8%	57.7%	56.6%	bf	bf = (as + aj) / h
Membrane 2 Concentration Factor	25.2	27.7	34.7	bg	bg = ((as + aj) / bl) / o
Overall Concentration Factor	38.3	23.3	26.0	bh	bh = ((as + aj) / bl) / a
Membrane 2 Extract 3	AL-02-2008-27	AL-02-2008-33	AL-02-2008-40		
qPCR Result, copies/mL	--	--	--	bi	From MRI Results Sheet
Sample & Vial Gross Weight, g	1.1695	1.1519	1.1615	av	From Test Run Sheet
Vial Tare Weight, g	1.0807	1.0465	1.0401	aw	From Test Run Sheet
Sample Weight, g	0.0888	0.1054	0.1214	ax	ax = av - aw
Number Copies	--	--	--	bj	bh = bg * ax
Membrane 3 % Efficiency	--	--	--	bk	bi = bh / y * 100%
Membrane 2 Extract 1, 2, & 3 together					
Total Sample Weight, g				bl	bl = ax + ar + ai
Membrane 2 % Efficiency				bm	bm = (bj + as + aj) / y * 100%
Total % Efficiency	--	--	--	bn	bl = (bj + as + aj) / h * 100%
Membrane 2 Concentration Factor				bo	bo = ((bj + as + aj) / bl) / o
Overall Concentration Factor	--	--	--	bp	bp = ((bj + as + aj) / bl) / a

Table C-2. Data Reduction for the DNA InnovaPrep Concentration Project (LWI 2008-01)

	High Level Runs			Line ID	
	Run 4	Run 5	Run 6		Source/Formula
Blank Extract Performed after Run	AL-02-2008-34	No Number			
qPCR Result, copies/mL	6.79E+07	--	--	ay	From MRI Results Sheet
Sample & Vial Gross Weight, g	1.1797	1.3415	--	az	From Test Run Sheet
Vial Tare Weight, g	1.0623	1.2647	--	ba	From Test Run Sheet
Sample Weight, g	0.1174	0.0768	--	bb	bb = az - ba
Number Copies	7.97E+06	--	--	bc	bc = bb * ay
% of Last Run	2.1%			bd	bd = bc / h * 100%

Table C-3. **Data Reduction for the DNA InnovaPrep Concentration Project (LWI 2008-01)**

	Repeat of Low Level Runs				Line	
	Run 12	Run 13	Run 14	Run 15	ID	Source/Formula
Feed	AL-02-2008-126	AL-02-2008-134	AL-02-2008-142	AL-02-2008-150		
- qPCR Result, copies/mL	6.86E+05	8.42E+05	7.82E+05	5.55E+05	a	From MRI Results Sheet
- Feed & Vial Gross Weight, g	16.5655	16.5971	16.4925	16.5121	b	From Test Run Sheet
- Vial Tare Weight, g	6.9659	6.9329	6.9410	6.9239	c	From Test Run Sheet
- Feed Weight, g	9.5996	9.6642	9.5515	9.5882	d	d = b - c
- Sample & Vial Gross Weight, g	1.2374	1.2211	1.2744	1.2802	e	From Test Run Sheet
- Vial Tare Weight, g	1.0426	1.0260	1.0783	1.0868	f	From Test Run Sheet
- Sample Weight, g	0.1948	0.1951	0.1961	0.1934	g	g = e - f
- Number Copies Fed	6.59E+06	8.14E+06	7.47E+06	5.32E+06	h	h = a * g
Membrane 1 Waste	AL-02-2008-127	AL-02-2008-135	AL-02-2008-143	AL-02-2008-151		
- qPCR Result, copies/mL	1.28E+06	2.78E+06	4.89E+06	3.35E+06	i	From MRI Results Sheet
- Sample & Vial Gross Weight, g	1.2479	1.2165	1.1845	1.2110	j	From Test Run Sheet
- Vial Tare Weight, g	1.0453	1.0641	1.0392	1.0469	k	From Test Run Sheet
- Sample Weight, g	0.2026	0.1524	0.1453	0.1641	l	d = b - c
- Number Copies	2.60E+05	4.24E+05	7.11E+05	5.50E+05	m	m = l * i
- % Lost to Membrane 1 Waste	3.9%	5.2%	9.5%	10.3%	n	n = m / h x 100%
Membrane 1 Permeate/Membrane 2 Feed	AL-02-2008-128	AL-02-2008-136	AL-02-2008-144	AL-02-2008-152		
- qPCR Result, copies/mL	2.07E+05	1.73E+05	1.14E+05	6.98E+04	o	From MRI Results Sheet
- Permeate & Vial Gross Weight, g	17.6977	17.0643	16.8286	16.5641	p	From Test Run Sheet
- Vial Tare Weight, g	7.5649	7.2497	6.9277	7.2446	q	From Test Run Sheet
- Permeate Weight, g	10.1328	9.8146	9.9009	9.3195	r	r = q - p
- Sample & Vial Gross Weight, g	1.2251	1.2265	1.1404	1.2264	s	From Test Run Sheet
- Vial Tare Weight, g	1.0320	1.0351	1.0437	1.0337	t	From Test Run Sheet
- Sample Weight, g	0.1931	0.1914	0.0967	0.1927	u	u = s - t
- Membrane 2 Feed Weight, g	9.9397	9.6232	9.8042	9.1268	v	v = r - u
- Number Copies in Permeate	2.10E+06	1.70E+06	1.13E+06	6.51E+05	w	w = o * r
- Membrane 1 % Efficiency	31.8%	20.8%	15.1%	12.2%	x	x = w / h
- Number Copies Fed	2.06E+06	1.66E+06	1.12E+06	6.37E+05	y	y = o * v
Membrane 2 Waste	AL-02-2008-129	AL-02-2008-137	AL-02-2008-145	AL-02-2008-153		
- qPCR Result, copies/mL	--	--	2.47E+03	--	z	From MRI Results Sheet
- Sample & Vial Gross Weight, g	17.2075	10.5172	16.5276	16.5641	aa	From Test Run Sheet
- Vial Tare Weight, g	7.2194	6.8888	6.9389	7.2446	ab	From Test Run Sheet
- Sample Weight, g	9.9881	3.6284	9.5887	9.3195	ac	ac = aa - ab
- Number Copies	--	--	2.37E+04	--	ad	ad = ac * z
- % Total Lost to Membrane 2	--	--	2.12%	--	ae	ae = ad / y x 100%

Table C-3. **Data Reduction for the DNA InnovaPrep Concentration Project (LWI 2008-01)**

	Repeat of Low Level Runs				Line ID	Source/Formula
	Run 12	Run 13	Run 14	Run 15		
Waste						
Membrane 2 Extract 1	AL-02-2008-130	AL-02-2008-138	AL-02-2008-146	AL-02-2008-154		
- qPCR Result, copies/mL	6.09E+06	6.04E+06	7.03E+06	2.13E+06	af	From MRI Results Sheet
- Sample & Vial Gross Weight, g	1.0948	1.1795	1.1880	1.1722	ag	From Test Run Sheet
- Vial Tare Weight, g	1.0440	1.0450	1.0722	1.0643	ah	From Test Run Sheet
- Sample Weight, g	0.0508	0.1345	0.1158	0.1079	ai	ai = ag - ah
- Number Copies	3.09E+05	8.12E+05	8.14E+05	2.30E+05	aj	aj = ai * af
- Membrane 2 % Efficiency	15.0%	48.9%	73.0%	36.1%	ak	ak = aj / y * 100%
- Total % Efficiency	4.7%	10.0%	10.9%	4.3%	al	al = aj / h * 100%
- Membrane 2 Concentration Factor	29.4	35.0	61.8	30.5	am	am = af / o
- Overall Concentration Factor	8.9	7.2	9.0	3.8	an	am = af / a
Membrane 2 Extract 2	AL-02-2008-131	AL-02-2008-139	AL-02-2008-147	AL-02-2008-155		
- qPCR Result, copies/mL	2.55E+06	1.07E+06	1.44E+06	5.63E+05	ao	From MRI Results Sheet
- Sample & Vial Gross Weight, g	1.1822	1.1531	1.1753	1.2002	ap	From Test Run Sheet
- Vial Tare Weight, g	1.0506	1.0437	1.0506	1.0539	aq	From Test Run Sheet
- Sample Weight, g	0.1316	0.1094	0.1247	0.1463	ar	ar = ap - aq
- Number Copies	3.36E+05	1.17E+05	1.80E+05	8.23E+04	as	as = ar * ao
- Membrane 2 % Efficiency	16.3%	7.0%	16.1%	12.9%	at	at = as / y * 100%
Membrane 2 Extract 1 & 2 together						
- Total Sample Weight, g	0.1824	0.2439	0.2405	0.2542	be	be = ar + ai
- Membrane 2 % Efficiency	31.4%	55.9%	89.2%	49.0%	au	au = (as + aj) / y * 100%
- Total % Efficiency	9.8%	11.4%	13.3%	5.9%	bf	bf = (as + aj) / h
- Membrane 2 Concentration Factor	17.1	22.1	36.4	17.6	bg	bg = ((as + aj) / bl) / o
- Overall Concentration Factor	5.2	4.5	5.3	2.2	bh	bh = ((as + aj) / bl) / a
Membrane 2 Extract 3	AL-02-2008-132	AL-02-2008-140	AL-02-2008-148	AL-02-2008-156		
- qPCR Result, copies/mL	--	1.62E+05	4.55E+05	2.57E+05	bi	From MRI Results Sheet
- Sample & Vial Gross Weight, g	1.1369	1.1676	1.1417	1.1535	av	From Test Run Sheet
- Vial Tare Weight, g	1.0386	1.0668	1.0377	1.0376	aw	From Test Run Sheet
- Sample Weight, g	0.0983	0.1008	0.1040	0.1159	ax	ax = av - aw
- Number Copies	--	1.63E+04	4.74E+04	2.97E+04	bj	bh = bg * ax
- Membrane 3 % Efficiency	--	1.0%	4.2%	4.7%	bk	bi = bh / y * 100%
Membrane 2 Extract 1, 2, & 3 together						
- Total Sample Weight, g	0.2807	0.3447	0.3445	0.3701	bl	bl = ax + ar + ai
- Membrane 2 % Efficiency	--	56.9%	93.4%	53.7%	bm	bm = (bj + as + aj) / y * 100%

Table C-3. **Data Reduction for the DNA InnovaPrep Concentration Project (LWI 2008-01)**

	Repeat of Low Level Runs				Line	
	Run 12	Run 13	Run 14	Run 15	ID	Source/Formula
Total % Efficiency					bl	bl = (bj + as + aj) / h * 100%
-	--	11.6%	13.9%	6.4%	bn	
- Membrane 2 Concentration Factor	--	15.9	26.6	13.2	bo	bo = ((bj + as + aj) / bl) / o
- Overall Concentration Factor	--	3.3	3.9	1.7	bp	bp = ((bj + as + aj) / bl) / a
Blank Extract Performed after Run	AL-02-2008-125	AL-02-2008-133	AL-02-2008-141	AL-02-2008-149		
qPCR Result, copies/mL	3.51E+05	6.57E+05	9.43E+05	2.94E+05	ay	From MRI Results Sheet
Sample & Vial Gross Weight, g	1.1478	1.1628	1.1499	1.1901	az	From Test Run Sheet
Vial Tare Weight, g	1.0391	1.0566	1.0478	1.0407	ba	From Test Run Sheet
Sample Weight, g	0.1087	0.1062	0.1021	0.1494	bb	bb = az - ba
Number Copies	3.81E+04	6.98E+04	9.63E+04	4.39E+04	bc	bc = bb * ay
% of Last Run	0.6%	0.9%	1.3%	0.8%	bd	bd = bc / h * 100%

*See previous tables for formulas or sources of data.

Leonard Wood Institute Research Project
Final Report

LWI Subaward Number:	LWI 16201
Project Title:	Demonstration of a Sample Preparation Method for Biological Detection Based on a Novel Membrane Fractionation Technology
Principal Investigator:	Andrew Page
Co-Investigator(s):	Pam Murowchick
Period of Research:	June 26,2008 – December 31, 2008

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1. Project Overview/Abstract

LWI funding was obtained to gather proof-of-concept data for processing of DNA in the InnovaPrep© system (formerly referred to as Hydraprep). The InnovaPrep is a novel membrane fractionation and particle concentration system developed by the InnovaPrep Division of AlburtyLab, Inc. of Drexel, MO. The system holds significant promise as a rapid sample preparation and concentration device for nucleic acids. Funding was requested for the purpose of developing methods and performing testing to determine the efficiency for (1) passage of DNA through a cleanup InnovaPrep membrane and (2) concentration of DNA in a final concentrating InnovaPrep membrane. Dr. Dorsey Newcomb was the Program Manager for LWI. Mr. Andrew Page was the overall project leader and primary investigator for AlburtyLab, Inc. and Mrs. Pam Murowchick and Mr. Page were the authors of this report. Technical questions may be directed to Mr. Page at (816) 619-3374 or via email to apage@innovaprep.com.

AlburtyLab contracted with Midwest Research Institute (MRI), of Kansas City, Missouri, to prepare a stock of *Bacillus globigii* (Bg), also known as *Bacillus atrophaeus*, and to lyse the material with a stand, commercially available bead beater. The lysed stock was delivered to AlburtyLab for processing with the dual stage InnovaPrep system. After processing with the dual-stage InnovaPrep system the samples were returned to MRI for analysis by quantitative PCR (qPCR). A total of 15 runs were conducted at two feed titer test levels. The efficiency for DNA passage through membrane 1 ranged from 9.0% to 31.8% for the low level test runs and from 74.4% to 148.4% for the high level test runs. DNA captured onto membrane 2 was recovered into nominal volumes of 100 microliters. Efficiencies ranged from 12.1% to 73.0% for the low level test runs. High level test runs were from 25.9% to 64.1%. The second set of low level test runs ranged from 15.0% to 73.0%. Overall concentration factors for processing through membrane 1 and 2 ranged from 1.2 to 9.0 for the low level test runs and between 39.1 and 45.8 for the high level test runs.

2. Project Schedule

This project was scheduled to be performed between June 26, 2008 and September 2008. The project was finished as scheduled with additional time taken for reporting. A no-cost time extension for reporting was granted by Dr. Paula Mihalcik, the original LWI Project Manager.

Table 1. Task Chart

Task	Completion Date
Project Initiation Date	6/26/08
Receive Hollow Fiber Filters	7/11/08
Receive Bg DNA stock from MRI	08/12/08
First Sample Set Submitted to MRI for Analysis	08/13/08
Receive 1st Sample Set qPCR Results	08/15/08
Receive 2nd Bg DNA stock from MRI	08/21/08
Second Sample Set Submitted to MRI	08/25/08
Receive 2nd Sample Set qPCR Results	08/27/08
Complete Technical and Summary Reports	12/31/08

3. Project Outcomes

In the fields of biodefense, food safety, water monitoring, and medical diagnostics, there is a significant push to move towards automated microbiological analysis using cutting-edge technology that provides fast results, better detection, and reduces overall costs. A wide range of these new, rapid microbiological detectors have appeared recently. They potentially provide more accurate and quicker analysis than was previously available. However, the use of these detectors is considerable limited by their small analysis volumes (e.g. less than 100 µL). For many applications, detection limits require significantly larger volumes to be analyzed. For nearly all pathogenic organisms the infectious dose is extremely low. Concentration of these organisms in the blood of an infected patient can be less than one organism per milliliter; a concern when compared to the most sensitive Polymerase Chain Reaction (PCR) detector's requirement of at least one organism in their traditional 100 µL analysis volume. This highlights the need to concentrate these organisms within the sample prior to detection, and this holds true whether detection is confirmed by classical cultivation methods or by employing the newest rapid microbiological methods. In recent years there have been revolutionary advancements in rapid microbiological methods; however, sample preparation methods to support these methods have remained relatively stagnant, and automated sample preparation systems are virtually non-existent for integration with these systems. Thus, while the detection itself may be automated, all or most of the sample preparation must still be performed manually in the laboratory. Various authors have reported on the lack of available sample preparation technologies.[1,2] According to the authors of the National Academy of Sciences publication *Sensor Systems for Biological Agent Attacks* sample preparation is the single most important challenge to be faced for detect-to-warn biological agent detection systems.[1] The same challenge faces those developing fully automated systems for detection of microorganisms in sample matrices specific to food safety, water monitoring, and medical diagnostics.

Microfluidics is often hailed as the optimal platform for developing automated sample preparation systems for integration with modern rapid microbiological methods. The benefits associated with miniaturization of bioanalytical techniques are well known.[3-5] These include reductions in the equipment size, shortened reaction times, and reduced component and reagent costs. Automated prototypes have been developed on microfluidic platforms that can perform cell separation, cell lysis, and nucleic acid purification from aqueous samples and a few systems have been developed for preparing nucleic acids from blood or blood products.[2,6,7] However, microfluidics alone do not provide an acceptable platform for performing automated sample preparation and concentration of large (e.g. greater than 1 mL) sample volumes. A solution is needed that allows nucleic acids to be prepared from volumes of greater than 1 mL and a large percentage of the target RNA or DNA to be concentrated into volumes that can be directly analyzed by rapid microbiological methods.

In the field of medical diagnostics, the most life threatening form of infection is that of the blood, referred to as bacteremia. During an infection organisms may be in low concentrations in blood (1-10 CFU/mL).[8] At present, culture of blood is the gold standard for diagnosing bacteremia, but depending on the pathogen involved, it may take more than 2 days before results are available.

Therefore, the clinical impact of blood cultures is limited.[9] PCR detection of bacteremia directly in blood samples, without prior cultivation, offers a rapid alternative to the blood culture method.[10] Limitations encountered with this approach include reduced sensitivity and reproducibility due to difficulties in handling large volumes (5-10 mL) of blood and increased complexity of equipment and protocols for nucleic acid extraction, PCR amplification, and detection. A more sensitive and more rapid determination of bacteremia would positively impact treatment options.

Although PCR is capable of detecting a single organism, protocol sensitivity may still be too low to warrant implementation of the assays in routine practice. The main issue is the small volume (typically 200 µL of blood) used in DNA isolation and subsequent PCR amplification. This can be compared to the 10 mL volume of blood required for a blood culture. The use of such a small volume of blood can lead to sampling error, especially in the case of low-grade bacteremia, which could be solved by processing a larger volume of blood for PCR. A rapid approach for concentrating microorganisms in 10 mL of blood into a volume adequate for PCR testing would alleviate this shortcoming. There have been a number of ways proposed to deal with this issue including both positive and negative immunoselection, various centrifugation methods, and column based separations. Shortcomings to these approaches include increased time for sample processing, extensive equipment requirements, reduced sensitivity, and protocol complexity. Although sensitive, PCR has not yet been fully accepted as a viable option for detection and identification of bacteremia.

In June, 2008, under LWI Subaward 16201, AlburtyLab was awarded a demonstration project to develop methods and generate proof of concept data for a novel automated sample preparation system for nucleic acids. The core technology in this system was recently developed by AlburtyLab, Inc. of Drexel, MO and Page Applied Research LLC of Kansas City, MO. Termed InnovaPrep, the technology is used to concentrate bacteria, viruses, or other particles of interest contained in a large liquid volume into a very small liquid volume thus, significantly lowering the level of detection. In addition to concentrating these particles, the processes and components developed for the InnovaPrep technology allow the system to efficiently fractionate particles by size, thereby removing larger particles and soluble components that can inhibit analysis. Through concentration, cell lysis, and purification of nucleic acids this system will produce analysis-ready nucleic acids for use in PCR detectors and other rapid, microbiological detectors.

The InnovaPrep technology was originally developed because a need was seen for a macro-to-micro interface between aerosol collectors and detectors in bioterrorism defense detection systems. The fully automated system is easily integrated with other fluidic systems and detectors. Particles of interest are captured onto a hollow fiber or other membrane. The captured particles are then extracted using a proprietary carbonated, ionic foam that efficiently recovers them into a liquid sample of significantly lower volume.

InnovaPrep Background

In bioterrorism defense detection systems an aerosol collector works to capture particles in the air and concentrate them into a liquid volume in the range of 1.5 to 15 mL. A small portion of this sample is then transferred directly to a detector which analyzes the liquid and determines if any dangerous aerosols have been collected. Advanced, rapid microbiological detectors used in

these systems are only capable of analyzing volumes from around 40 to 200 µL of liquid at a time; thus 2% or less of a 10 mL sample from the collector is analyzed. Any remaining fluid is either archived or dumped to waste. The InnovaPrep technology was developed as a method for delivering a higher percentage of the particles found in a sample to the detector thus improving the method detection limit by orders of magnitude.

The concentration system works by pumping the liquid sample into a concentration cell that contains one or more hollow fiber membrane filters. Pre-treatment of samples is performed if needed. Several microvalved fluid and gas paths allow the sample to be introduced to the cell and concentrated on the fiber(s) in dead-end configuration, while the fluid and particles not captured are passed through the fiber into the permeate liquid. The membrane surface area, pore size, material, transmembrane pressure, membrane loading process, and other factors can be tailored to optimize the concentration. Following concentration onto the fiber, further treatment (such as washing) can be performed until the captured particles are eluted using a novel wet foam elution method. The final sample volume is chosen to match the analytical technique to be used. A process diagram for the InnovaPrep concentration cell (patent pending) is shown in Figure 1.

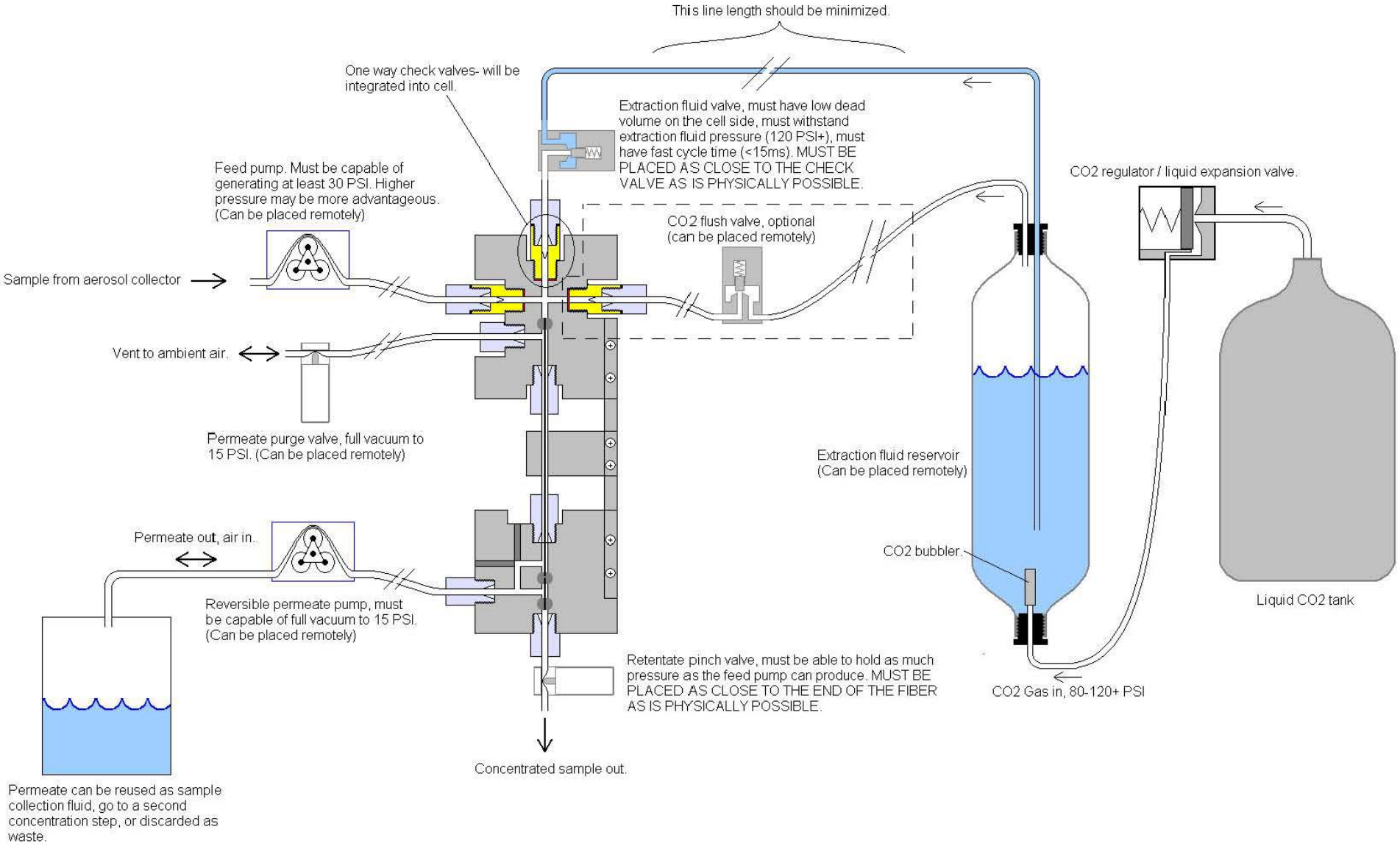

Figure 1. InnovaPrep Process Diagram

Recovery of the collected organisms from hollow fibers is performed with buffered carbonated foam. The foam is produced by rapidly dispensing an extraction solution containing a surfactant or other foaming agent from a high pressure carbon dioxide atmosphere through a capillary (or other means of agitation) and into an expansion zone. High quality foam is produced and swept through the hollow fiber extracting the particles. This novel method, developed by AlburtyLab and Page Applied Research, is referred to as wet foam elution.

Foam has unique properties that make it a superior method for extraction of hollow fiber concentration cells. Extraction with aqueous foam has significant advantages over extraction with aqueous solutions. Possibly the most important aspect of using foam for extraction is the advantage gained by expanding the extraction fluid to many times its original volume. Because most of the extraction fluid is needed for filling the internal void volume of the hollow fibers, rather than performing the actual extraction at the fiber surface, it is important to keep the internal volume of the concentration cell small. However, it is difficult to get sufficiently high throughput rates while maintaining a sufficiently small internal volume. Due to the potential for clogging, there is also a lower limit to the internal diameter of individual hollow fibers. In the InnovaPrep, liquid is expanded approximately five times, allowing a concentration cell to be approximately five times the size, for five times the throughput, while maintaining similar efficiencies. Alternatively, it is possible to use smaller pore size hollow fibers, which will have a lower flux but can provide higher efficiencies and can make it possible to concentrate much smaller particles, such as viruses or toxins. AlburtyLab has begun construction of an InnovaPrep system that will allow expansion of the extraction fluid by up to approximately twenty times, resulting in an approximate increase of volume throughput of twenty times over using an aqueous extraction solution, while maintaining similar efficiencies.

Foam has been used and studied extensively for use in semiconductor cleaning, radioactive particle removal, and for decontamination of biological agents. Foams are also frequently used during hydrocarbon exploration, with enhanced oil recovery being the most common use. Due to the high viscosity of foams, they exhibit reduced channeling thereby sweeping more oil out of porous media.[11] This same characteristic makes foam ideal for extraction of multiple fiber concentration cells. It is well known that channeling or non-uniform flow distribution takes place in hollow fiber modules that contain multiple fibers.[12-14] Channeling is dependent on inlet manifold design, Reynolds number, pressure drop, and other issues such as irregularity in fiber diameter.[12-13] In short, channeling is difficult to control and is almost certainly a factor during recovery of organisms from multiple fiber concentration units with aqueous extraction fluids.

The flow of foams in small channels is a complex subject. Several research teams have recently studied this subject and determined that uniform foams in a capillary exhibit plug flow and self-lubricate with a very narrow lubricating layer thickness on the order of 10 μm or less.[15-16] The foam moves as a rigid body lubricated by water generated by breaking foam at the wall[15]. Because core flow is absent and the lubricating layer is thin, the foam is able to act at the fiber surface to sweep away the captured particles. Further, energy created by the bursting bubbles may enhance particle removal from the hollow fiber surface.

The majority of the bubbles in the InnovaPrep carbonated extraction foam burst soon after release from the concentration cell, returning the foam to a liquid within 30 seconds and allowing

for transfer to the detector as an aqueous sample. Buffering the foam mitigates the potential for damage to nucleic acids during concentration. The use of foam to extract the hollow fiber concentrator units produces significantly higher recoveries of organisms and allows for the use of greater fiber surface area, thereby decreasing the time needed to process samples.

In addition to concentrating these particles, the processes and components developed for the InnovaPrep technology allow the system to efficiently fractionate particles by size, thereby removing larger particles and soluble components that can inhibit analysis. Through membrane fractionation, cell lysis, and concentration this system will produce analysis-ready nucleic acids for use in PCR detectors and other rapid, microbiological detectors.

System Testing

A flow chart of the experimental setup used for this project is shown below in Figure 2. Samples to be analyzed are marked with the orange numbers 1 through 7. To reduce sample analysis costs not all samples were analyzed for each test run. Samples included (1) a sub-sample of the lysed input material, (2) concentrated waste from membrane 1, (3) a sub-sample of the permeate from membrane 1, (4) permeate waste from membrane 2, (5) concentrate consisting of the concentrated DNA in the first extraction of membrane 2, and (6) a second extraction from membrane 2. A third extraction from membrane 2 (7) was archived. All analyses were performed in triplicate.

Feed samples and extraction fluid contained 0.025% Triton and were prepared with DNA grade water. Extraction fluid was buffered with 25 mM Tris buffer. The first membrane (Membrane 1) served as a cleanup step by removing cellular debris and other particles too large to pass through the 0.2 µm pore size. The second membrane (Membrane 2) captured the DNA on the surface while particles smaller than the 100 kD pore size passed through. The DNA was then extracted into a final concentrate of 100 µL nominal volume.

Membrane 1 contained a single 0.2 µm mixed cellulose ester membrane from Spectrum Laboratories, Inc. The effective length of this membrane is approximately 72 mm with an internal diameter of 0.6 mm. Because an air space is left at the bottom end of the fiber the length of fiber covered by liquid during operation is approximately 64 mm. This provides a utilized fiber surface area of 1.2 cm^2 with a total internal volume of 21 µL. Membrane 2 served as the concentration membrane and contained a single 100 kD polysulfone membrane (General Electric (GE) Healthcare, Inc). The effective length of this membrane is approximately 234 mm with an internal diameter of 1 mm. During operation, the length of the membrane covered by fluid is approximately 178 mm. This provides a utilized fiber surface area of 5.6 cm^2 and a total internal volume of 190 µL. Both of these membranes were removed from standard off-the-shelf hollow fiber units and repotted into the units built by AlburtyLab.

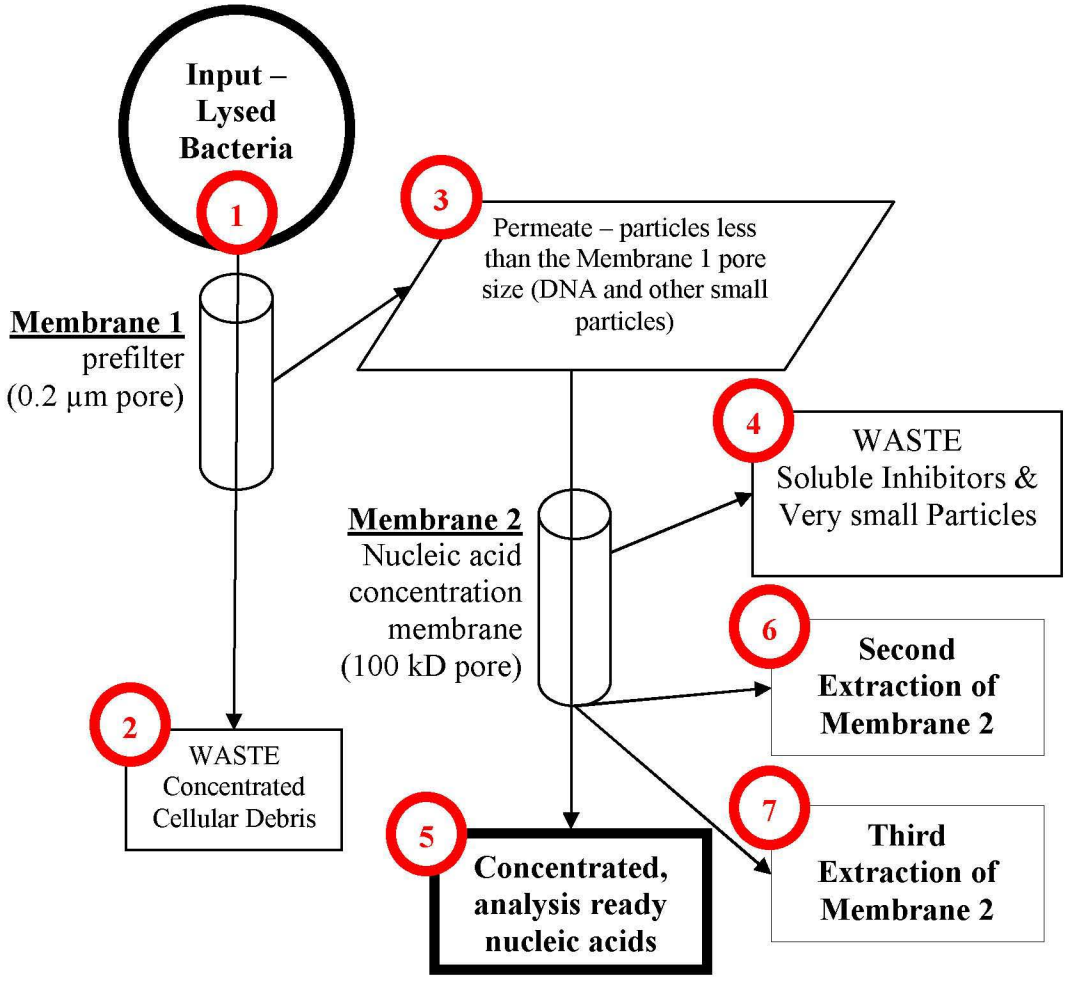

Figure 2. Flow Chart of the Test

Membrane 1 flux was estimated to be in the range of 350 to 550 µm/second with transmembrane pressures of 9 to 15 psi. Pressure was only applied to the feed side. The permeate side of the hollow fiber was open to atmospheric pressure. Time required for feed throughput plus extraction of the membrane ranged from approximately 3.5 minutes to 5 minutes. Some of the later runs (Run 9 and later) used a small amount of 0.025% Triton to flush the feed line and a foam flush through to the permeate side to assist in removing DNA that had been retained on the internal surfaces of the system.

Membrane 2 feed pressures were substantially higher than those used for Membrane 1. Membrane 2 was used on an InnovaPrep unit with a pressure transducer feed back for controlling the feed pressure. The set point used for all test runs was 20 psi. The permeate pump was run a full rate, so that the transmembrane pressure was probably approaching 30 psi. Feed throughput plus extraction times ranged from 5.5 minutes to 10.5 minutes. The estimated flux was 33 to 75 µm/second. Extraction fluid pressure was set to 100 psi and the extraction fluid time was set to 20 milliseconds.

The high level feed suspensions were prepared by serial diluting the DNA preparation received from MRI with 0.025% Triton to a final dilution of 1 to $5x10^2$. This provided a concentration of $3.95x10^7$ copies/mL or approximately 198 ng/mL as determined by the qPCR results. The low level feed suspensions were prepared using the same method with a final dilution of the MRI DNA preparation of 1 to $5x10^4$. Results from the qPCR analysis determined a low level feed suspension concentration of $5.13x10^5$ copies/mL or approximately 2.56 ng/mL. A second set of low level feed tests were conducted using a feed with a final dilution of 1 to $4.5x10^4$. An aliquot of the feed stock from each of the second set of low level test runs was analyzed by qPCR. The concentrations ranged from $5.55x10^5$ to $8.42x10^5$ copies/mL or approximately 2.78 to 4.21 ng/mL.

The analysis results combined with the sample weights were used to calculate the efficiencies and concentration factors across each column and across the entire Dual Column InnovaPrep System. Table 2 summarizes this data. The average of the runs for each of the conditions is provided in Table 3.

The efficiency that DNA passed through membrane 1 ranged from 9.0% to 14.7% for the initial low level test runs and from 74.4% to 148.4% for the high level test runs. After making some operational changes to the system four additional low level test runs were performed. The efficiencies for membrane 1 ranged from 12.2% to 31.8%.

The efficiencies for the first extraction of membrane 2 ranged from 12.1% to 26.5% for the initial low level test runs. High level test runs were from 25.9% to 64.1%. The second set of low level test runs ranged from 15.0% to 73.0%. Overall concentration factors for processing through membrane 1 and 2 ranged from 1.2 to 4.1 for the initial low level test runs. The high level test runs ranged from 39.1 to 45.8 and the second set of low level test runs ranged from 3.8 to 9.0.

Table 2. Summary of InnovaPrep DNA Testing

	Low Level Runs			High Level Runs			Repeat of Low Level Runs			
	Run 3	Run 7	Run 8	Run 4	Run 5	Run 6	Run 12	Run 13	Run 14	Run 15
Feed										
qPCR Result, copies/mL	5.13E+05	5.13E+05	5.13E+05	3.95E+07	3.95E+07	3.95E+07	6.86E+05	8.42E+05	7.82E+05	5.55E+05
Feed Weight, g	9.95	9.79	9.97	9.80	9.92	9.87	9.60	9.66	9.55	9.59
Number Copies Fed	5.10E+06	5.02E+06	5.11E+06	3.88E+08	3.92E+08	3.90E+08	6.59E+06	8.14E+06	7.47E+06	5.32E+06
Membrane 1 Permeate/Membrane 2 Feed										
qPCR Result, copies/mL	6.04E+04	7.53E+04	4.70E+04	6.00E+07	3.33E+07	2.97E+07	2.07E+05	1.73E+05	1.14E+05	6.98E+04
Permeate Weight, g	9.83	9.79	9.77	9.59	9.77	9.77	10.13	9.81	9.90	9.32
Membrane 1, % Efficiency	11.6%	14.7%	9.0%	148.4%	82.9%	74.4%	31.8%	20.8%	15.1%	12.2%
Number Copies Fed	5.82E+05	7.22E+05	4.50E+05	5.64E+08	3.19E+08	2.85E+08	2.06E+06	1.66E+06	1.12E+06	6.37E+05
Membrane 2--Extract 1										
qPCR Result, copies/mL	9.13E+05	2.08E+06	6.23E+05	1.81E+09	1.55E+09	1.70E+09	6.09E+06	6.04E+06	7.03E+06	2.13E+06
Sample Weight, g	0.086	0.092	0.087	0.081	0.114	0.107	0.051	0.135	0.116	0.108
Number Copies	7.89E+04	1.91E+05	5.45E+04	1.46E+08	1.75E+08	1.82E+08	3.09E+05	8.12E+05	8.14E+05	2.30E+05
Membrane 2, % Efficiency	13.6%	26.5%	12.1%	25.9%	55.1%	64.1%	15.0%	48.9%	73.0%	36.1%
Total, % Efficiency	1.5%	3.8%	1.1%	37.6%	44.7%	46.7%	4.7%	10.0%	10.9%	4.3%
Membrane 2 Concentration Factor	15.1	27.6	13.3	30.1	46.5	57.3	29.4	35.0	61.8	30.5
Overall Concentration Factor	1.8	4.1	1.2	45.8	39.1	43.1	8.9	7.2	9.0	3.8
Membrane 2--Extracts 1 & 2 together										
qPCR Result, copies/mL	2.47E+05	1.12E+06	1.98E+04	1.28E+09	3.83E+08	3.60E+08	2.55E+06	1.07E+06	1.44E+06	5.63E+05
Sample Weight, g	0.146	0.111	0.103	0.103	0.132	0.108	0.132	0.109	0.125	0.146
Membrane 2, % Efficiency	19.7%	43.6%	12.6%	49.4%	70.9%	77.7%	31.4%	55.9%	89.2%	49.0%
Total % Efficiency	2.3%	6.3%	1.1%	71.8%	57.7%	56.6%	9.8%	11.4%	13.3%	5.9%
Membrane 2 Concentration Factor	8.2	20.7	6.3	25.2	27.7	34.7	17.1	22.1	36.4	17.6
Overall Concentration Factor	1.0	3.0	0.6	38.3	23.3	26.0	5.2	4.5	5.3	2.2
Membrane 2--Extracts 1, 2, & 3 together										
qPCR Result, copies/mL	--	--	--	--	--	--	--	1.62E+05	4.55E+05	2.57E+05
Sample Weight, g	--	--	--	--	--	--	--	0.101	0.104	0.116
Membrane 2, % Efficiency	--	--	--	--	--	--	--	56.9%	93.4%	53.7%
Total % Efficiency	--	--	--	--	--	--	--	11.6%	13.9%	6.4%
Membrane 2 Concentration Factor	--	--	--	--	--	--	--	15.9	26.6	13.2
Overall Concentration Factor	--	--	--	--	--	--	--	3.3	3.9	1.7
Blank Extract Performed after Run										
qPCR Result, copies/mL	--	--	--	6.79E+07	--	--	3.51E+05	6.57E+05	9.43E+05	2.94E+05
% of Last Run	--	--	--	2.1%	--	--	0.6%	0.9%	1.3%	0.8%

Table 3. Average Efficiencies and Concentration Factors for InnovaPrep DNA Testing

	Low Level Runs		High Level Runs		Repeat of Low Level Runs	
	Average	SD	Average	SD	Average	SD
Membrane 1, % Efficiency	11.8%	2.9%	101.9%	40.5%	20.0%	8.7%
Membrane 2--Extract 1						
Membrane 2, % Efficiency	17.4%	7.9%	48.3%	20.0%	43.3%	24.3%
Total % Efficiency	2.1%	1.5%	43.0%	4.8%	7.5%	3.5%
Membrane 2 Concentration Factor	18.7	7.8	44.6	13.7	39.2	15.3
Overall Concentration Factor	2.4	1.5	42.6	3.3	7.2	2.4
Membrane 2--Extracts 1 & 2 together						
Membrane 2, % Efficiency	25.3%	16.3%	66.0%	14.8%	56.4%	24.2%
Total % Efficiency	3.2%	2.7%	62.0%	8.5%	10.1%	3.2%
Membrane 2 Concentration Factor	11.7	7.8	29.2	4.9	23.3	9.0
Overall Concentration Factor	1.5	1.3	29.2	8.0	4.3	1.4
Membrane 2--Extracts 1, 2, & 3 together						
Membrane 2, % Efficiency	--	--	--	--	68.0%	22.1%
Total % Efficiency	--	--	--	--	10.7%	3.9%
Membrane 2 Concentration Factor	--	--	--	--	18.6	7.1
Overall Concentration Factor	--	--	--	--	2.9	1.1

Since performing the test runs described in this report, additional paper study and laboratory work were performed outside this program by AlburtyLab. The goal of this was to acquire further information that may assist in improving the observed efficiencies in future work. Journal articles were found that pertain to the passage of DNA and other particles through hollow fiber membranes. The majority of the research cited in these articles was performed with the goal of improving the efficiency with which DNA passes through 0.2 μm and smaller pore size membranes during membrane sterilization of pharmaceutical production of plasmid DNA. This is a very recent research area and it is currently focused on production of large quantities of high purity plasmid DNA for gene therapy and DNA-based vaccines. The research that has been performed is applicable to the InnovaPrep system; however the majority of these data is for DNA concentrations that are one or more orders of magnitude greater than those used for this study. It is possible to extrapolate from some of these data back to the concentrations used for this study, but it is not entirely clear whether this is meaningful for all of the parameters discussed below.

Multiple authors have investigated the parameters that control whether DNA passes through or is retained by a membrane. The parameters that have been observed to have the greatest effect are membrane pore size, size of DNA, total concentration of particles in solution, solution conductivity or salt concentration, pH, and flux rate or trans-membrane pressure. For a given pore size it is possible to significantly affect the retention or passage of DNA by controlling the pH, salt concentration, or trans-membrane pressure. In general, an increase in any of these three parameters causes an increase in passage of DNA. An overview of research that has been performed in this area is shown below in Table 4.

DNA was passed through Membrane 1 efficiently at the high concentrations but not at the lower concentrations. From review of the data presented in the referenced articles it also appears that Membrane 1 should have passed the DNA with high efficiencies. Buffering the pH of the feed solution and adding a salt should reduce charge interactions that may have reduced the efficiency of Membrane 1 and Membrane 2. The addition of the salt will act to increase passage of DNA through Membrane 2. Therefore it will be necessary to reduce the pore size and reduce the transmembrane pressure for Membrane 2 in order to reduce passage of DNA.

.Table 6. Summary of DNA Fractionation Research

Latulippe et al.[1,2,3]	3 kb circular plasmid	Flat/stirred cell–regen. Cellulose, PVDF (0.22 µm)	100 kD, 300 kD, 1000 kD, 0.22 µm	100 kD–18 to 105 300 kD–20 to 125 1000 kD–4 to 80 0.22 µm–6 to 135	100 kD–0.5 to 5.8 300 kD–0.5 to 4.1 1000 kD–0.5 to 1.7 0.22 µm–not report.	1, 10, 40, 150 NaCl	7.7	250; 750; 2,500
Hirasaki et al.[4]	3 kb circular plasmid, 46 kb & 154 kb DS lin.	BMM Dead-end hollow fiber regen. cellulose	15 nm (~200 kD), 35 nm (~475 kD)	-	0.5, 1.0, 1.9, 3.9	Tris/ EDTA	7.5	2,150; 5,300; 11,250; 15,000
Higuchi et al.[5]	2kb to 23 kb SS & DS lin.	BMM Dead-end hollow fiber ($30cm^2$) regen. cellulose	15 nm (~200 kD)	1.1 to 2.2	3.9	Tris/ EDTA	7.4 to 8.0	10,000; 25,000; 50,000; 75,000; 100,000
Arkhangelsky et al.[6]	9.5 kb circular plasmid	Flat/stirred cell cellulose acetate, PES	20 kD	-	7.25, 14.5, 29.0, 43.5, 58.0, 72.5	10, 50, 100 NaCl	3,4,5,6,7,8,9, 10, 11	330
Kong et al.[7]	6, 20, 29, 33, 72, 116 kb Circular plasmid	Flat PVDF (0.22 µm), PES (0.2 µm)	0.22 µm, 0.2 µm	17, 33, 133, 383		Tris/ EDTA, 150 NaCl	8.0	25,000 to 100,000
InnovaPrep – Stage 1	Total-bead beat Bg	Dead-end hollow fiber mixed cellulose ester	0.2 µm	300 to 500	9 to 15	None – 0.025% Triton	No buffer	2.56 to 198
InnovaPrep – Stage 2	Total-bead beat Bg	Dead-end hollow fiber polysulfone	100 kD	33 to 75	25 to 30	None – 0.025% Triton	No buffer	2.56 to 198

4. Overall Assessment of Project

Advanced systems that provide automated purification and concentration of nucleic acids from aqueous samples are virtually non-existent. The InnovaPrep technology holds significant promise for automated preparation of nucleic acids for analysis by PCR or other methods. By passing nucleic acids through a large pore membrane, cellular debris and other large particles can be removed from the sample. The sample can then be captured onto a second smaller pore size membrane where the nucleic acids are captured and the sample fluid is removed and replaced with a small volume of clean analysis-compatible buffer.

A two-stage InnovaPrep system, for DNA cleanup and concentration was tested during this project. While efficiencies for DNA processing were lower than may be deemed acceptable for implementation into some current biosampling and detection systems, the concentration factors for all ten test runs were above 1x and thereby provide a net increase in sample concentration to the detector. After several operation changes were made an additional three low level test runs were performed. These test runs provided an average concentration factor of 7.2x with a standard deviation of 2.4x.

In one way the InnovaPrep clearly separates itself from all other currently available nucleic acid sample preparation systems due to the ability to pass a fluid through the system in a continuous flow, retaining the nucleic acids for subsequent elution into a small final volume. This continuous-flow, batch process makes the process an innovative tool for many applications.

5. Way Ahead

AlburtyLab believes that significant improvement in the efficiency of the InnovaPrep system can be made with minimal operational changes to the system. Seven key changes have been identified that are expected to provide significant improvement to the system.

1. Feed should be 25 mM Tris, 0.15 M NaCl, pH 7.2, 0.05% Triton X-100, and 1 mM EDTA or similar. Salt concentration should be kept above about 50 mM.
2. Replace existing Norprene® peristaltic tubing sets with platinum cured silicon tubing.
3. In order to reduce pulsing and formation of bubbles and foam in the fluid line use the smallest peristaltic tubing size that will deliver the required flow rate and use improved peristaltic pumps.
4. Use the shortest practical tubing length and if possible flush tubing with foam or liquid after each step to reduce losses.
5. For the stage one membrane use 0.65 micrometer, 1 mm ID, polysulfone membrane with approximately 16 cm^2 surface area. Operate the membrane at pressures up to approximately 2 psi transmembrane pressure. At 2 psi a flux of approximately 37 ml/min is possible. Higher pressures are acceptable and should not significantly reduce efficiencies but may increase the rate of membrane flux decline.
6. Efforts should be made in the system design to keep all fluid path surfaces completely covered with fluid whenever possible. This is important to keep DNA from contacting dry or partially dry surfaces and binding to those surfaces. It is especially difficult to keep the outer surface of membrane one covered with fluid. As an alternative to keep the surfaces covered in fluid it may be possible to flush the surfaces with clean feed solution or extraction foam.
7. For the stage two membrane, use a 50 kD pore size, 0.5 mm id, polysulfone membrane with approximately 26 cm^2 surface area. Operate the membrane at a maximum transmembrane pressure of approximately 6 psi. A flux of approximately 2 mL/min is possible at 6 psi.

As an initial step AlburtyLab plans to implement these changes and retest the system. We plan to use a PicoGreen® (Invitrogen/Molecular Probes, Inc.) fluorescent dye labeling method to determine the concentration of DNA in the feed, permeate, and concentrate. As part of this testing we plan to evaluate the ability of the system to remove soluble and insoluble materials. A final test will involve retesting the system with low levels of DNA with analysis by qPCR.

6. Financial Summary (Final Voucher)

A final overall itemized voucher is provided below. The voucher format used throughout the project period of performance is the final voucher format. This attachment is an Excel File named LWI-Phase 0 Final Voucher LWI 16201 123108 DA.xls.

7. Endorsement

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APPROVED FOR ALBURTYLAB, INC.

Andrew E. Page
Principal Investigator

Pamela S. Murowchick
Co-Investigator

David S. Alburty
President

December 31, 2008

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